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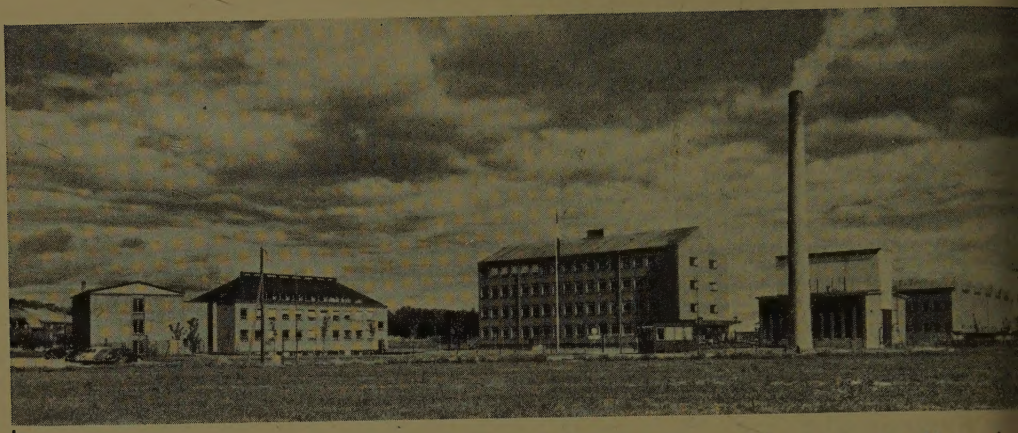

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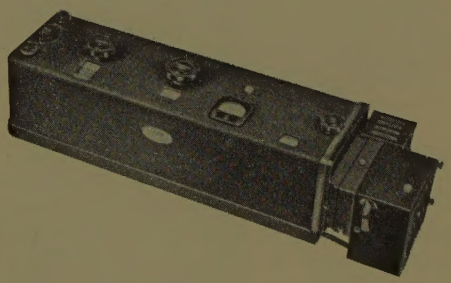

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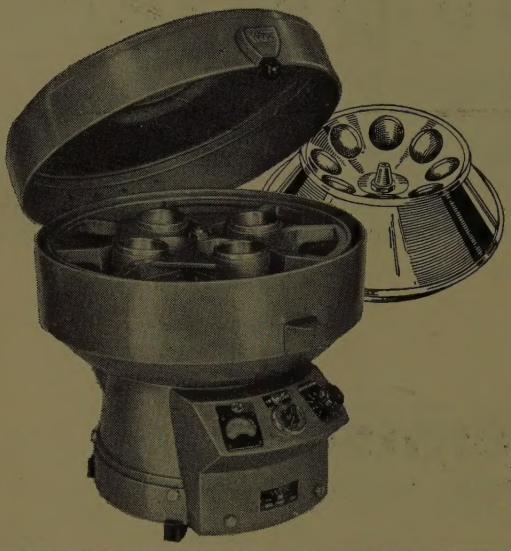

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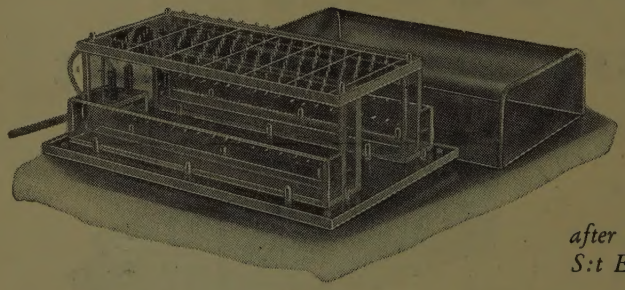

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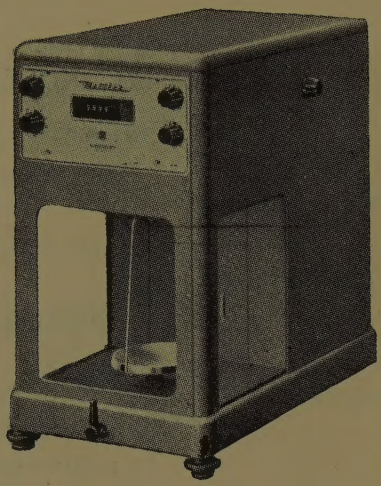

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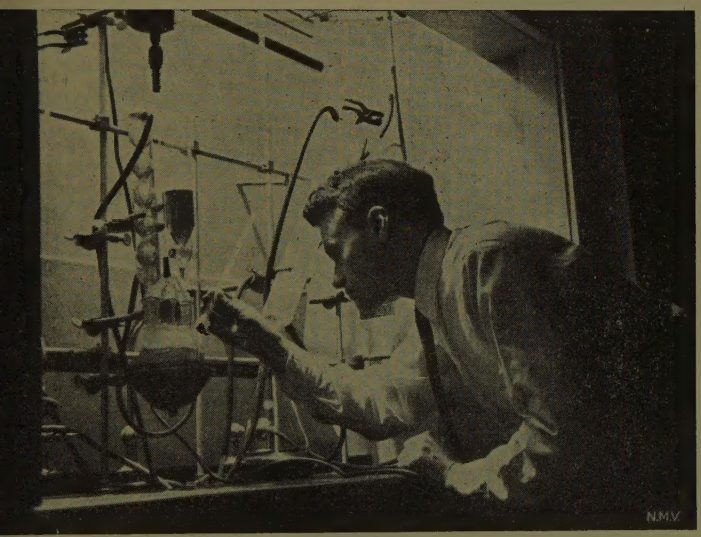

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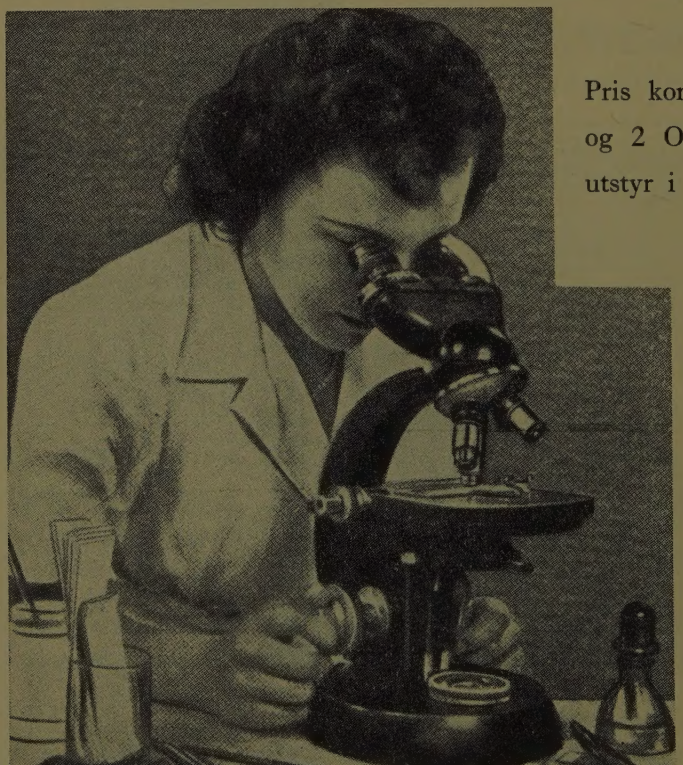

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THE INFLUENCE OF TIME ON REABSORPTION FROM URINE IN COLON

PERIMENTAL STUDIES ON THE CONDITIONS FOLLOWING URETEROCOLIC ANASTOMOSES II[1,2]

By M. PERS

From the Surgical Department of the Finsen Institute, Copenhagen, and the Medico-Physiological Institute, University of Copenhagen, Denmark

(Received for publication Dec. 23, 1954)

atients with ureterocolic anastomoses hloremic acidosis, elevation of the urea level and dehydration are nt occurences (Ferris & Odel, 1950), e transfer of ions through the mucous ane of the colon seems to be a factor ed in the development of this lyte imbalance (Annis & Alexander, Parsons; Pyrak, Powell, Reed & 1952; Rosenborg, 1953).

author (1954) has previously studied erations observed in urine specimens d for a two-hour period in an isolated t of the colon in a dog. Several ions could be drawn from these gations, but the relations between the ents of the urinary constituents and uld not be controlled by the method employed. Hence, some questions still remain unanswered.

Concentrated urine specimens were seen to have increased in volume when retained in the colon for two hours. A return of fluid to the organism may, however, already have commenced before the specimen was withdrawn, and a mere recording of the volume after two hours is therefore no reliable measure for the quantities of water that may pass into urine in the colon. The question may accordingly be raised: *How long will concentrated urine specimens retained in the colon continue to increase in volume?*

The net reabsorption of chloride from all hypotonic urine specimens studied was observed to be almost complete within two hours. It could not be settled, however, whether complete reabsorption was first attained from the most dilute urine specimens or first from the less dilute ones. According to Goldschmidt & Dayton (1919),

ed by grants from King Christian X's ion, Weimann's Bequest and P. A. Brandt's

hnical assistance by Mrs. B. Bendixen.

the absorption of chloride from simple solutions of saline is delayed at low concentrations, but no investigations have been made to determine whether the same is true for the reabsorption of chloride from urine specimens. Another question arising is therefore: *Is a high percentile net reabsorption of chloride attained later from the most dilute urine specimens than from the less dilute ones?*

An ambi-directional permeability of the colon for chloride was demonstrated by the same writers, and a net colonic secretion of chloride was found to occur at concentrations of saline below 27 mE/l and 37 mE/l at normal and elevated plasma chloride concentration respectively. If similar threshold values exist for the passage of chloride to and from urine in the colon, a colonic net loss of chloride might be obtainable in patients with ureterocolic anastomoses by provoking a high urine flow. We may therefore ask: *Does a net secretion of chloride ever occur into urine in the colon?*

The absolute amounts of urea reabsorbed within two hours from different urine specimens were almost identical in the previous experiments. This result may, however, be a fortuitous finding due to the choice of a two-hour period. The problem is accordingly: *Is urea always reabsorbed at a fairly constant rate from urine in the colon?*

The passage of hydrogen ion equivalents to the organism from urine in the colon depends partly on a reabsorption of H^+ as such or as NH_4^+, and partly on a secretion of bicarbonate. As the bicarbonate secreted may later be reabsorbed, the total amount of H^+ added to the organism may again diminish after a certain time. Fur clarification on this point is desirable: *which time is the maximum addition hydrogen ion equivalents to the organ reached?*

To answer the above questions exp ments have been carried out, in which gradual changes in urine volume contents of urinary constituents in the c were followed at short intervals durin fairly long experimental period.

METHODS

A segment of the colon of a dog was iso in order to study the alterations in de amounts of urine introduced. Details previously been reported (Pers, 1954).

To be able to assess the gradual alteratio volume, inulin was added to the urine, as concentration of inulin was supposed to inversely proportional to the volume. (While present experiments were being carried out, B & Rupe (1953) proposed phenolsulphonphth for a similar purpose.) As "inulinoid" values of about 0.05 per cent were observe concentrated urine specimens, an initial conce tion as high as 3 per cent in the urine was advantageous.

The experiments were extended over (Nos. 1—10) or four hours (Nos. 11—20). of urine was introduced, and a 5 ml sample withdrawn for analysis every 40 minutes. was determined by a modification of diphenylamine method (Bojesen, 1952), chloride, sodium, ammonia, and urea by proce previously described (Pers, 1954).

It soon appeared that not all the inuli troduced could be recovered in the samples fluid evacuated *plus* wash water. The incon recovery of inulin might be due to 1) abso through the mucous membrane, 2) descompo of inulin, or 3) ineffective final evacuatio washing.

1) Absorption of inulin was studied i following way: 50 ml of a 3 per cent soluti

as introduced and retained in the gut for utes. In two experiments the recovery was 5 per cent, *i. e.* about 200 mg inulin was not d in the evacuated fluid + wash water. 35 mg inulin was found in the urine pro- the animal during the experimental period. t day 200 mg inulin (corresponding to the not recovered from the gut) was infused iously at a constant rate during a similar (160 minutes). The urine produced by the hin this period contained 105 mg inulin, only 35 mg in the previous experiment. fference suggests that about one-third of in that was not recovered had been absorbed

he possibility that inulin was converted in to substances that evaded analysis could be t of account, because inulin-containing ns of urine withdrawn from the gut and d at 37° C, in ten cases showed no fall in in values during the experimental period.

he effectiveness of the final evacuation trolled by introduction of 50 ml of inulin followed by immediate withdrawal and . The recovered inulin amounted to 100 ± cent in ten such experiments. If, on the and, inulin was allowed to remain longer in *e. g.* two hours, a binding of inulin to the was demonstrable. After a two-hours xperiment the gut was washed thoroughly lin-free fluid was introduced. An increasing of inulin appeared in this fluid during the 0 minute, indicating that inulin was

subtotal recovery of inulin thus seemss to en due partly to a certain, minor absorption the mucous membrane and partly to on on mucus. It must be admitted that vith inulin small amounts of water and constituents may have been located in the but it was, however, decided to make cor- for the total amount of inulin that was not d in the actual experiments.

amount of inulin in the gut diminished he experiment from that introduced ($v_o \cdot c_o$) total amount recovered (R). The volume s therefore been calculated from the inulin ration c_t at the time t, not by the simple equation $v_t = \frac{v_o \cdot c_o}{c_t}$, but by $v_t = \frac{v_o \cdot c_o - A}{c_t}$, where the correction (A) is the portion of the total loss of inulin during the experimental period (T), having already occurred at the time t, *i. e.* $A = (v_o \cdot c_o - R) \cdot \frac{t}{T}$. The correction presupposes that the loss of inulin occurred at a constant rate.

In the actual experiments the percentage of inulin recovered was between 70 and 100 per cent. The recovery was lower, the more the urine volume had been reduced at the end of the experiment. The explanation seems to be that under such conditions a higher percentage will be adsorbed on the mucus and reabsorption will be favored by a high concentration gradient.

RESULTS

Diagrams of three representative experiments (Figs. 1, 2 and 3) illustrate the observed sequence of events in dilute (group 1, specific gravity 1006—1014), moderately concentrated (group 2, specific gravity 1014—1026), and highly concentrated urine specimens (group 3, specific gravity 1026—1034).

Water movements

The total concentration of urinary constituents seems decisive for the direction and rate of the net water movements (Fig. 4).

Water was reabsorbed throughout the experimental period from hypo- and isotonic urine specimens (group 1), while slightly hypertonic specimens (group 2) at first drew water into the intestinal lumen and later returned water to the organism, apparently when sufficient amounts of osmotically active substances had been reabsorbed. Highly concentrated specimens (group 3) drew

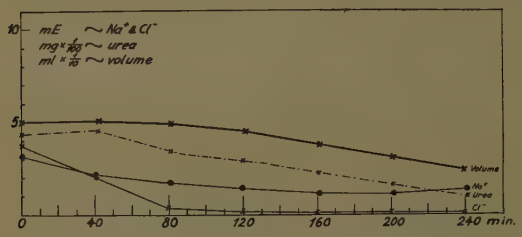


Fig. 1. Urine specimen with specific gravity 1012. Note the almost complete net reabsorption of chloride. (Exp. no. 18.)

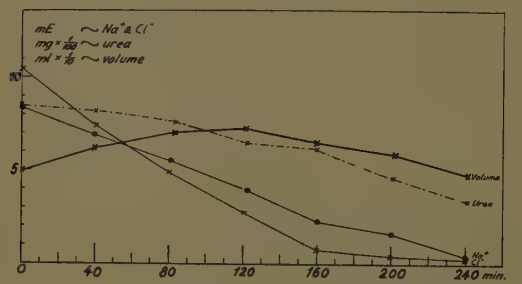


Fig. 2. Urine specimen with specific gravity 1020. Water is at first drawn into the colon and later returned to the organism. (Exp. no. 12.)

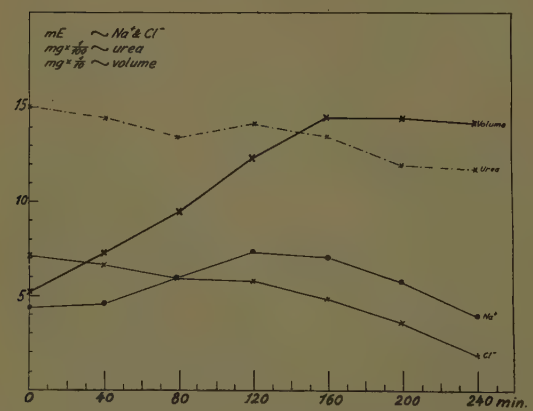


Fig. 3. Urine specimen with specific gravity 1026. A net secretion of sodium is demonstrated. Urea is reabsorbed at a similar rate as from more dilute specimens. (Exp. no. 16.)

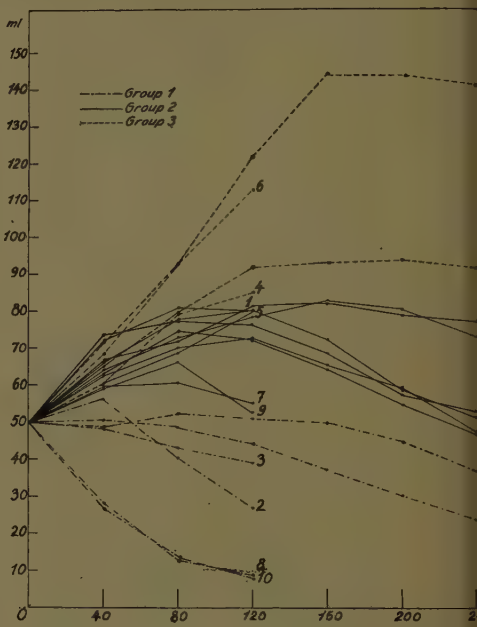


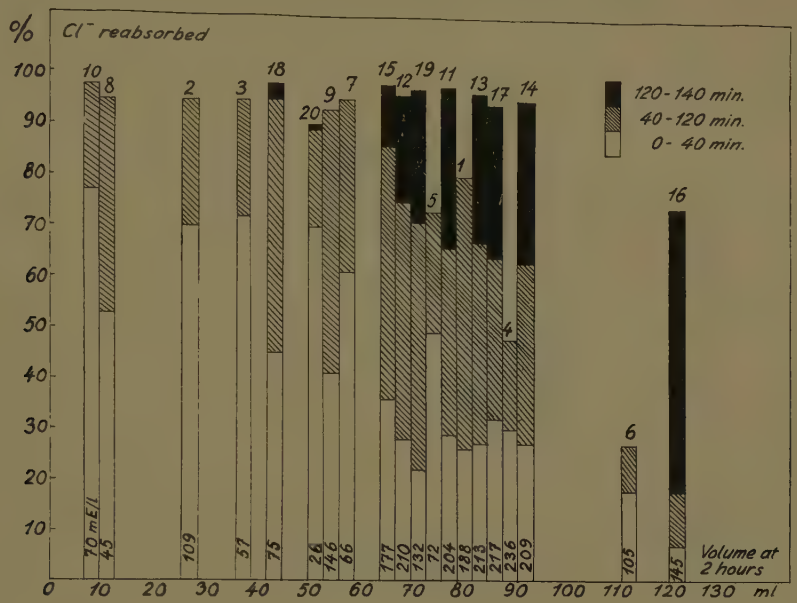
Fig. 4. Changes in the volume of different specimens (50 ml) introduced into an isolated segment of the colon.

water into the lumen at a fast rate during the first two or three hours, and no return of water to the organism was observed within four hours.

Chloride

In patients with ureterocolic anastomosis the percentile net reabsorption of an urinary constituent from the colon is a measure of the percentage of the renal excretion of the constituent, which becomes inefficient. For chloride this percentage has been calculated from the experimental results after 40, 120 and 240 minutes (Fig. 5).

It is seen that a high percentile net reabsorption of urinary chloride is reached more rapidly, the more dilute the specimen



5. The percentile net reabsorption of chloride from urine specimens retained for 40, 120 and 240 utes in the colon. The urine volume at two hours has been used as a relative expression for the l osmotic activity in the urine specimens employed. The original concentrations of chloride are indicated at the base of the columns.

chloride concentrations lower than t 7 mE/1 (as observed during experi- s with specimen from group 1 and 2) r was reabsorbed at a faster rate than ide. Consequently the chloride concen- on increased, but a net secretion of ide was never seen.

Urea

ie absolute net reabsorption within each inute period was determined. Average es were calculated for all three groups pecimens. The results are shown in 6 which also includes the general ge values for the total experimental ds.

appears that urea was reabsorbed at a constant rate, especially when judging from the general average values. It may be remarked that the slightly increased reabsorption observed in group 2 and 3 during the two last periods may have been due to the fact that the back-diffusion of urea from the concentrated specimens by that time no longer was inhibited by the water movement (cf. Fig. 4). The possibility of a certain decomposition of urea to NH_3 must also be considered, however.

Hydrogen ion equivalents

The amounts of hydrogen ion equivalents added to the organism from urine in the colon are with good approximation calculable by the difference between the net reabsorption of chloride and that of sodium (Pers, 1954). On this basis the hydrogen

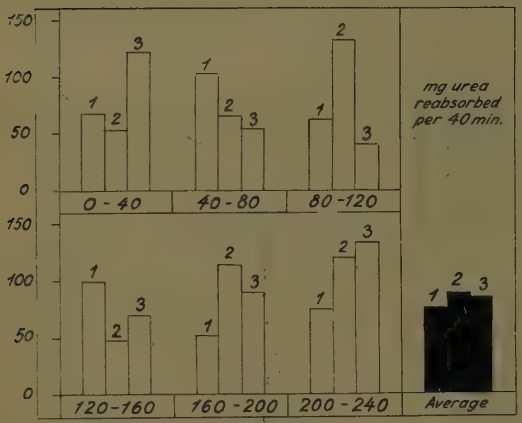


Fig. 6. The absolute net reabsorption of urea within the individual 40-minute periods. Average values for each group of specimens (1, 2 and 3). General average indicated in black.

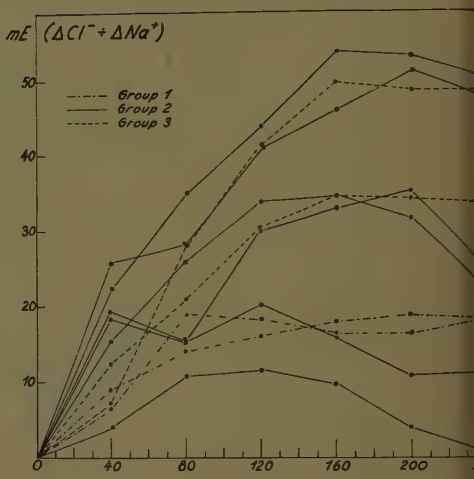


Fig. 7. Addition of hydrogen ion equivalents t organism. Estimations from the differ between the net reabsorption of chloride and of sodium ($\Delta Cl^- - \Delta Na^+$). Note the presen a maximum in the experiments with speci included in group 2.

ion equivalents added to the organism have been determined at intervals of 40 minutes during the four-hour experiments (Fig. 7).

It is evident that the main addition of hydrogen ion equivalents occurred during the first two hours, and it is worth noting that a marked reduction usually was observed later in the cases where moderately concentrated urine specimens were introduced (group 2). The dilute and the highly concentrated specimens (group 1 and 3) showed no appreciable changes later in the experimental periods.

DISCUSSION

The highest percentile net reabsorption of chloride was seen to be reached first from the most dilute urine specimens, and no net secretion of chloride was demonstrable. The results achieved by Goldschmidt & Dayton (1919) in studies based on simple solutions of saline in the colon therefore do not a to the passaage of chloride to and urine in the colon. Hence, they canno recently suggested by Mitchell & (1954), explain the absence of hy chloremia in patients with uretero anastomoses who had a large intak water.

Urea being returned at a fairly con rate, to all appearance independently concentration or time, a high percentile reabsorption of urea may be expected dilute urine produced with a high rat urine flow.

An ample intake of water seems, how to be the only means by which these pat may combat dehydration, as the movements into concentrated urine s mens in the colon will abolish the w

ng effect of renal oliguria in response
ıreatening dehydration.

he additional load caused by recircula-
of urea and electrolytes will prevent
ssive concentration of the tubular
e. Patients with ureterocolic anasto-
es will therefore hardly be able to
luce urine specimens so concentrated as
e classified here as group 3. For that
e reason it may also be questioned
ther urine corresponding to the most
e specimens (group 1) may be secreted.
urine commonly secreted by patients
ureterocolic anastomoses is therefore
t likely of the type classified here as
ıp 2.

egarding the experimental results, with
ial reference to group 2, both the loss
vater through the mucous membrane of
colon (Fig. 4) and the addition of
rogen ion equivalents to the organism
ı urine in the colon (Fig. 7) appear to
reached their maximum values within
ıt two hours. Obviously, the present
lts, obtained from a study of urine
imens introduced as a single sample are
fit for an exact evaluation of the trans-
to and from urine entering continuous-
Roughly they suggest, however, that in
nts with ureterocolic anastomoses an
interval of a few hours between evacuations of the colon is the least favorable as regards the transfer of water and hydrogen ion equivalents.

CONCLUSIONS AND SUMMARY

A method is described by which the gradual changes in the volume of urine retained in an isolated segment of the colon may be measured.

We may conclude from the results of 20 experiments that the volume will decrease if the specific gravity of the urine is below 1014. If between 1014 and 1026, the volume will increase for approximately two hours, but later fluid is returned to the organism. In more concentrated specimens such a return of fluid has not been observed within four hours.

Extremely low values for the contents of chloride may soon be reached in the retained urine, especially in the most dilute specimens. A net secretion of chloride into the urine in the colon has not been observed.

Urea is reabsorbed at a fairly constant rate, irrespective of concentration or time.

A time for maximum addition of hydrogen ion equivalents to the organism has been demonstrated. Under the stated experimental conditions this maximum was attained after approximately two hours.

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A NEW TESTTUBE METHOD FOR Rh GROUPING WITH THE USE OF INCOMPLETE Rh ANTIBODIES (SALINE/TRYPSIN METHOD)

A PRELIMINARY REPORT

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e supply of test serum containing anti-gglutinin active in physiologic saline is d and sometimes not sufficient to cover equirements of routine Rh-grouping. he other hand the supply of sera ining incomplete Rh-antibodies, even with a high titer, is good and therefore ing methods utilizing the power of sera to agglutinate Rh-positive red n albumin or serum have been devised. esearch laboratories preference is still to the standard test tube method using spension of red cells in physiologic and an agglutinating test serum active ine, but even there it is now sometimes sary to use incomplete Rh-antibodies e test tube method. For this purpose us technical procedures have been ed, of which probably the best hitherto ble is the so-called saline-albumin od. Unfortunately this modification of tandard test tube method has certain vantages, the most important of which

ne saline-albumin method requires two tra steps for every test tube (sucking f the saline solution and addition of umin solution).

ne final reaction in the saline-albumin ethod may sometimes be false owing to the same serological sources of error as those inherent in the rapid slide method (Broman, Löw & Ottow, 1954).

The standard test tube method is probably the most reliable for grouping material mainly from patients and only to a minor extent from registered donors or pregnant women. This, together with the availability of serum containing incomplete Rh- antibodies, called for a modification of the standard test tube method permitting the use of incomplete Rh-antibodies, but without the disadvantages of the saline-albumin method.

Morton & Pickles (1947) have shown that trypsin renders D-positive red cells agglutinable, even in physiologic saline, by sera containing incomplete anti-D. This observation marked an important step in the routine demonstration of Rh-antibodies in serum. Since then various proteolytic enzymes, especially trypsin and papain, have been studied for the effect on red blood cells.

Serum rapidly and markedly inhibits the proteolytic power of the enzyme. The method described below avoids this disadvantage. An exact amount of trypsin is added and followed by buffer solution to provide optimal pH environments for the enzymatic effect. The amount of trypsin

selected was such that the trypsin might act upon the red blood cells for about 20—30 minutes before it is inhibited by the serum. The sample should then be able to stand for 2 hours without the development of any panagglutinability of D-negative red blood cells.

The absolute and relative concentrations given below are to be regarded as preliminary. Further investigations are in progress to determine *inter alia* optimum trypsin effect and optimum trypsin inhibition. Investigations are also planned regarding the effect of the size of the drop and the concentration of the blood suspension as well as the effect of storage at different temperatures on the prepared trypsin-serum mixture. Other problems receiving attention are whether the addition of the patient's serum to the blood suspension influences agglutination and whether storage has any effect on the agglutinability of red blood cells, as judged by the method described.

The *trypsin solution* used was prepared from Trypure Novo (Manufacturers: Novo Terapeutisk Laboratorium A.B.), which is supplied as a dialysed and freeze-dried sterile preparation. Its proteolytic activity, as measured by Anson's hemoglobin method, is given as 20—25 Anson trypsin units per gram (1 Anson unit corresponds to about 250 000 Armour units). 0.05 g of this preparation was dissolved in 5 ml of 1/20 N hydrochloric acid. The solution was found to tolerate one month's storage at + 4° C without appreciable loss of its effect.

The *buffer solution* consisted of M/10 phosphate buffer with a pH of 7.7.

The *test serum* contained incomplete Rh-antibodies with a titer of 1:64—1:256, as determined by the albumin method. Dilution, when necessary (see below), was carried out with physiologic saline in connection with the addition of trypsin solution. The serum was stored at — 24° C use.

The *blood cells* were suspended in 2—3 p concentration in physiologic saline. The bloo used for routine work were not washed.

The test serum was prepared immediately use. 0.4 ml of buffer solution and 0.15—0.20 the trypsin solution described above were ad 0.2 ml of serum thoroughly mixed. The prepa was then ready for use.

The same test tubes and wooden blocks used as for routine grouping. One small (0.02 ml) of the test serum mixture was in each tube, after which an equally large (0.02 ml) of blood cell suspension was added tube was then shaken sufficiently to insure intermixture. The sample was placed in the mostat at 37° C for 2 hours after which the was read. The examination of the tubes below is more difficult than in the standar tube method, because the primary sedimenta not followed by agglutination until the tryps begun to act. However, once one has familiar with the method, reading of the from below usually offers no difficulties routine work the specimen is sucked up Pasteur pipette and examined macroscopical microscopically. For purposes of control series of unknown cells was extended to inc definitely D-positive and a definitely D-ne cell sample.

As a preparatory measure the serum to b was first examined for its power to aggl D-positive blood cells that had been treate trypsin in accordance with the method de by Pickles. This examination was carried an ordinary dilution series. The purpose measure was to check whether the serum use tolerate threefold dilution necessitated method. Some sera were found to be most if diluted more than 1:4. In these cases th were diluted to a suitable extent before addi the buffer solution and trypsin solution. necessary to treat every serum separately, composition then found suitable could be us daily requirements. However, the serum

be used the same day it was prepared; even stored overnight at —20° C it lost much of ivity.

is method has now been used for about nths in the examination of some 400 specimens, mainly from hospitalised nts, to a certain extent from pregnant en (not hospitalised) and to a minor t from registered blood donors. We d with the examination of random les in short series. Recently, however, xaminations were carried out in paral- ith routine tests, use being made of tinating anti-D serum active in physo- saline and supplied by the State Rh atory, Stockholm. Parallel series were with serum prepared at Malmö Blood sfusion Service in the manner described e. The actual results obtained by both ods, as well as the size and firmness of gglutinates, were in good agreement. agglutinates in the parallel series (with ared serum) were often larger and e all firmer than in the other series. instance was a specimen found to be gative in the routine series and D- ve in the parallel series or *vice versa*. addition to the routine tests, a number pecimens have been collected from nts with diseases in which blood ing tests are known to give false or iable results. Thus, specimens were ined from five patients with malignant nogranulomatosis, from one with blastic leukemia, from one with aplastic ia treated for 3 years with 116 blood fusions, from one with myeloma, and a number of patients with an erythro- sedimentation rate of more than 100 r (Westergren). The specimen from one of the patients with lymphogranulomatosis and from the patient with myeloblastic leukemia were found to be D-negative. In all of these cases the reactions were clear-cut and in no instance did the results obtained with the routine serum differ from those noted with the prepared serum. It is intended to complete the investigation with blood cells that are Rh-negative and direct Coombs-positive. In these cases one might expect a falsely positive agglutination, since some of the auto-immune-antibodies occurring in hemolytic anemia can be demonstrated by treatment with trypsin.

Twelve different anti-D-sera containing incomplete antibodies with a titer of 1:64—1:256, as measured by the albumin method, were examined in the manner described. Despite a titer of 1:256, two of these sera gave poorer reactions than the others. This might tentatively be ascribed to a more pronounced inhibitory effect of these sera on trypsin. All of the other sera treated showed the same good agreement as the serum tested first.

It is to be presumed that other anti-Rh sera with some other incomplete antibody (anti — C, —E, —c, —e, *etc.*) might be prepared in the same way and used in the method described above. In order to check this possibility, a serum containing incomplete anti-c was prepared in a series of 10 random specimens, and in one specimen from a person belonging to blood group 0 Rh +, type $Rh_1 Rh_2$. Here, too, distinctly positive and distinctly negative results were obtained and with control sample agglutination was slight but distinct. The possibility of using this serum and others is receiving further attention.

SUMMARY

A method is described for utilizing sera containing incomplete Rh-antibodies in Rh-grouping by the saline/trypsin method. A detailed description is given of the preparation of serum with trypsin and buffer solution. 400 specimen from a hos clientele were examined by this method by the routine test tube method. The re obtained by both methods were in pe agreement.

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PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

I. CLINICAL FEATURES AND INVESTIGATIONS ON THE HEMOLYTIC TENDENCY

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roxysmal nocturnal hemoglobinuria H) is a rare disease. Since Marchia- s publication in 1928, less than 100 have been reported.

patient with this disease has been ved and treated by splenectomy in men Hospital. The blood from the nt was subjected to investigations with d to the hemolytic tendency before and the operation (cfr. also the second part is paper by Hellem and Skaug).

e characteristic feature of this disease hemolysis which occurs during sleep, g a dark hemoglobin-containing urine. rule, the daytime urine has a normal

e actual cause of the hemolysis is un- n, but some mechanisms may be stated. e fact that the blood turns acid during because of reduced lung ventilation hat this acidifying process takes place mably where the blood circulates y — such as in the spleen — does not nce the erythrocytes in normal subjects. tients with PNH, this increased acidity hemolysis. This fact forms the basis Hams's test (1937, 1939) which is ve for the diagnosis. In this test the rocytes are mixed with serum to which is added hydrochloride acid to pH of approximately 6.6. Erythrocytes from patients with PNH are thereby hemolyzed, normal erythrocytes are not.

It has been emphasized that the hemolytic process is subjected to variations so that hemolysis may occur when the patient is awake. On the other hand, the nocturnal hemolysis may disappear for shorter or longer periods of time.

In addition to the fact that the erythrocytes cannot resist increased acidity, it is also found that a serum factor, possibly several factors (Clap, Williams & Mende, 1952) may be implicated in the hemolytic mechanism. This (or these) factor appears normally, not only in the sera from patients with NPH. The factor is thermolabile (is destroyed by heating to 56° C) and is retarded by addition of anticoagulants (citrate, oxalate and heparin — the latter in concentrations above 1/15 000 (Jordan, 1935).

It has been emphasized that this factor is a complement (Ham and Dingle, 1939). Crosby and Dameshek (1950) are of the opinion that this is not the case. On the basis of Jordan's findings that the anticoagulants have a retarding effect, Crosby and Dameshek have claimed that the serum factor has

Table I. *Hemolysis tests and pH measurements in blood from a patient with P before and after splenectomy.*

Test	Tp. and time of incubation		Before operation		After operat	
	Tp. °C	Time Hrs.	Hemo-lysis	Change of pH during incubation	Hemo-lysis	Change duri incuba
PNH erythroc. + acidif. norm. serum	37	1	++		++	
PNH erythroc. + acidif. PNH serum	37	1	++		++	
Norm. erythroc. + acidif. norm. serum	37	1	–			
Norm. erythroc. + acidif. PNH serum	37	1	–		–	
PNH blood (defibr.)	37	5	++	7.41—7.21	–	7.44—
PNH blood (defibr.)	37	18	++	7.41—7.22	++	7.44—
Norm. blood (defibr.)	37	5	–	7.41—7.21		
Norm. blood (defibr.)	37	18	–	7.41—7.20		
PNH blood (defibr.)	20	24	++	7.41—7.33	++	7.45—
Norm. blood (defibr.)	20	24	–	7.40—7.37		
PNH erythroc. + normal serum	37	1	(+)		(+)	
Norm. erythroc. + PNH serum	37	1	–		–	
PNH erythroc. + inactivated norm. serum ..	37	1	–		–	
PNH erythroc. + inactivated PNH serum ...	37	1	–		–	
PNH erythroc. + inactivated acidif. norm. serum	37	1	(+)		(+)	

a great similarity with accelerator globulin (Seegers) or proaccelerin — factor V (Owren), but this conception, however, has not been proved.

Serum from patients suffering from PNH shows no hemolytic effect on normal erythrocytes. Normal erythrocytes transfused to persons with PNH have normal survival time. On the other hand, erythrocytes from PNH transfused to normal persons have a considerably shorter survival time than normal, except when the recipient is a premature infant. Here the transfused erythrocytes have a longer or normal survival time. The interpretation has been that the premature baby has not fully developed the serum factor whi necessary for the hemolysis (Dacie & I 1943 and Dacie & Mollison, 1949).

When whole blood, serum or plas infused into a patient with PNH, hemolytic tendency is increased. Thi be prevented if the erythrocytes are w before the infusion.

The usual fragility test of the red cells is normal, however all evi indicates that the disease is prim associated with the erythrocytes.

CASE REPORT

The patient was a 40 year old Lapp w Her symptoms started in the spring of 195 progressive weakness. In the fall she was

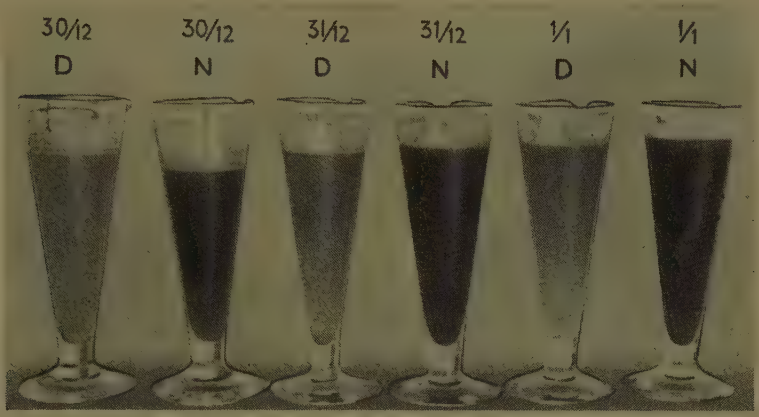

Fig. 1.

a hospital for anemia (Hemoglobin 38 % ~ 100 ml of blood) and was treated with blood usions. Following the transfusions the urine l bloody and the patient was jaundiced. The urine occurred during the night, the color lmost normal during the day.

e patient was then transferred to Drammen tal for further examination. The diagnosis was verified by Ham's and other tests e I). The urine from night and day was graphed — Fig. 1.

e patient was treated with suspensions of d erythrocytes — with and without ACTH. course was variable, however, and in little than one year her condition became serious was apparent that other measures had to be — if possible — to prevent exitus.

mentioned, the acidifying process of the has particularly been thought to take place spleen. It would therefore theoretically be to expect a favorable influence of removal e spleen. However, the results of this ion had never previously proved successful, for one case reported by Bo Anderson .

the basis mentioned above, splenectomy was med February 27, 1953 (Dr. K. Liavaag). immediate result was good. The massive lobinuria disappeared and the general on of the patient improved considerably.

The hemolysis tests, which were carried out for diagnostic purposes, were now repeated.

The principles of the methods applied can be classified in three groups:

1. Erythrocytes from patients suffering from PNH are hemolyzed by adding acid serum (pH approximately 6.6).
   In the present investigation acidified serum was prepared according to the directions published by Ham: 1.9 ml normal serum plus 0.1 ml N/3 HCl. If Ham's test is positive it means conclusively that the patient suffers from PNH.
   To the same group belong also methods which are based on the fact that blood becomes acid by standing either at room temperatur or at 37° C in an incubator. The production of lactic acid is strong enough to make the milieu sufficiently acid so that PNH erythrocytes are hemolyzed.
2. Erythrocytes from PNH patients are hemolyzed when mixed with normal serum and kept in an incubator at

37° C for a *short* period of time (1 hour). In this test the effect of the active serum factor or factors is demonstrated.

3. Erythrocytes from PNH are not hemolyzed if the serum used has been inactivated by being heated to 56° C. The hemolysis does not occur even if the inactivated serum is acidified. — This test underlines the importance of the serum factor or factors.

Methods based upon the principles, mentioned in groups 2 and 3 are, however, less reliable than those in group 1.

Defibrinated blood was used. If was ascertained that the control blood (from normal individuals) had the same blood type characteristics as that of the patient. pH was measured with a Radiometer pH-meter.

The results are tabulated in Table I. Each result represents several parallel tests. As observed, the conclusive tests are positive both before and after the splenectomy. The only difference is that after the operation the defibrinated blood did not show hemolysis after 5 hours, in an incubator, while strong hemolysis had occurred at this point before operation.

This finding indicates some retardation of the hemolytic tendency since there was no significant difference in the pH values 7.21 and 7.25 respectively. It is interesting to note that this result is supported by the results of the investigations with P^{32} as far as the tendency goes.

During the manipulation in connection with the various tests after the operation, the erythrocytes appeared to be more fragile than was the case arlier. An ordinary fragility test did not verify this impression.

However,the following examination sho that a minor hemolysis occurs during usual coagulation process. —

Blood from the patient and a control indiv was taken by venipuncture into three centr tubes: 1. Ordinary dry tube. 2. Heparin-t (dry film) tube (approximately 0.1 mg hepar blood). 3. The blood was here collected unde

The samples were taken *in duplo* — an tubes were left standing for 1¼ hours to s complete retraction of the clot. The he tube and the oil tube were then centrifuged.

With a Pasteur pipette the ordinary serum heparin plasma and the oil serum were col for analyses. The results were verified by rep examinations.

The results are shown in Table II.

Table II. *The hemoglobin conten in mg per 100 ml*[1]

	Serum (regular coagulation)	Heparin plasma	se
1. M.G. (Patient with NPH)	60	14	
U.B. (Control)	6	6	

[1] Bing & Bakers method (1931).

It seems evident: 1) that some hemo has taken place during the usual coagul process in the blood of the PNH patien that the erythrocytes really are more fr than normal, 2) that oil in some way pre this hemolysis, presumably by retardin coagulation process, 3) that heparin *in* has not retarded this hemolysis more tha 4) that to judge the degree of the hemo in the blood of the PNH patient, it is n sary to collect the blood in heparin-tr tubes or under oil. Otherwise, one m led astray regarding the condition *in vi*

ble III. *Hemolysis tests and pH measurements in blood from a patient with PNH shortly after splenectomy and 8 month later.*

	Time and tp. of incubation		Shortly after splenectomy		8 months later	
	Tp °C	Time hrs.	Hemo-lysis	Change of pH during incubation	Hemo-lysis	Change of pH during incubation
erythroc. + acidif. norm. serum	37	1	++		+	
erythroc. + inactivated norm. serum..	37	1	(+)		−	
blood (defibrinated)	37	5	−	7.41—7.21	−	7.44—7.23
— —	37	18	++	7.41—7.22	(+)	7.44—7.19

COMMENTS

has been demonstrated by Hellem and g that the uptake of radioactive phos-us in the erythrocytes from the sur-ling medium occurs more rapidly after peration than before. It has also been nstrated that the hemolytic tendency, ured on defibrinated blood at 37° C, s after splenectomy than before.

th facts indicate that splenectomy has d an alteration of the hemolytic anism. However, the results cannot be letely explained by the theoretical ıption which was the basis for the tion, namely that splenectomy should ve an area which favored hemolysis gh an increased accumulation of carbon de by a particularly slow passage of . This procedure should decrease the lytic tendency, but should not affect ıte of uptake of P^{32}.

e preoperative investigations with P^{32} ed that there was a splitting of ATP ıpanied by a liberation of inorganic hate after 60 minutes in the blood of tient with PNH. This did not occur in lood of the control. This abnormality e intra-erythrocytic phosphate-carbo-hydrate turnover can be interpreted as an enzymatic dysfunction, as it is known that the phosphate-carbohydrate turnover rate is generally enzymatically controlled.

The agreement between the hemolytic and the P^{32} studies both before and after the splenectomy indicates that there probably is a close relationship between the hemolytic mechanism and the intra-erythrocytic phosphate-carbohydrate turnover. It has previously been shown by Crosby & Dameshek (1950) that the serum factor has enzymatic properties. It therefore appears reasonable that the enzymatic conditions in the blood which prevail during PNH should be subjected to further studies. The exact role of the spleen in the hemolytic mechanism during PNH is still obscure.

SUMMARY

1. A description is given of the hemolytic condition in a patient with Paroxysmal Nocturnal Hemoglobinuria (PNH) before and after splenectomy.
2. An increasing retardation of the hemolytic tendency is found after splenectomy, both clinically and by means of hemolysis tests on defibrinated blood.

3. It is also shown by *in vitro* experiments that the usual coagulation process causes some hemolysis in blood from the patient. This hemolysis can be prevented by collecting the blood in heparin-treated tubes or under oil.
4. There is agreement between the hemolytic and the P^{32} studies and it appears that the splenectomy has affected both the hemolytic tendency and the uptake of P^{32} in the erythrocytes. This fact seems to indicate that there probably is a close relationship between the hemolytic mechanism and the intra-erythrocytic phosphate-carbohydrate turnover in PNH.

Added in proof:

Eight months later the patient was operated on for gallstones with cholecystitis. She tolerated the operation well, and the postoperative course was uneventful. Investigations on the hemolytic tendency were repeated and a still further decrease was noted — as shown in Table III.

In January 1955 the condition of the patient was still good, but now and then she becomes anemic and gets blood transfusions, however, with increasing intervals.

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PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

II. PERMEABILITY AND PHOSPHATE TURNOVER IN THE RED BLOOD CELLS

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(Received for publication May 21, 1954)

ıe permeability and the phosphate ɔver in the red blood cells have been ed in a patient with Paroxysmal ırnal hemoglobinuria (PNH). We to the first part of this paper where ıer & Veflingstad give an account of clinical features of this disease and rt investigations regarding the hemo- tendency.

THE PERMEABILITY

Methods

ɔut 40 cc of blood were drawn from the ıt. Clotting was prevented by heparine. A amount of carrier-free P^{32} was added and ımple incubated at 37° C in a water bath. The e was carefully shaken during the experiment gassed with atmosperic air. Samples were rawn in most cases after 0, 30, 60, 120, 180 300 min. incubation. The samples were im- ıtely cooled to 0° C in icewater in order to nt exchange of ions between plasma and scles. Plasma was separated by centrifuga- 0.1 cc of plasma or in some cases 0.1 cc of ed cell mass was transferred to small alu- n discs, dried and the radioactivity deter- in a Geiger counter. The samples were kept ı order to prevent self absorbation. According ıe hematocrit value and the radioactivity ssed in counts per min./ml, the increase of ty in the corpuscles is calculated and expres- ıs per cent of the total activity which etically could be obtained when all the radio- activity of 1 ml blood is accumulated within the corpuscles.

The amount of radioactivity which can migrate from the plasma and be taken up the corpuscles must depend on the corpuscle/ volume per ml blood, *i. e.* the hematocrit value.

In a few experiments the uptake of radio- activity was directly determined in the red cell mass obtained by centrifugation. In such experiments a small contamination with plasma-about 3 per cent — is unavoidable.

The permeability of the corpuscles was investigated in 5 controls, one of which was a daughter of the patient. Neither by clinical nor by laboratory examination could the disease be traced in her (Ham's test etc.). The patient was investigated 3 times before splenectomy and once one month after.

The results are shown in Fig. 1.

A delayed uptake of radioactivity in the corpuscles was found in the patient's blood. After splenectomy a delayed uptake was still found, but less marked than before the operation. The migration of phosphate from plasma to the corpuscles must depend on the following 2 factors:

1. The permeability of the membrane.
2. The phosphate turnover within the red cells, — or a combination of both.

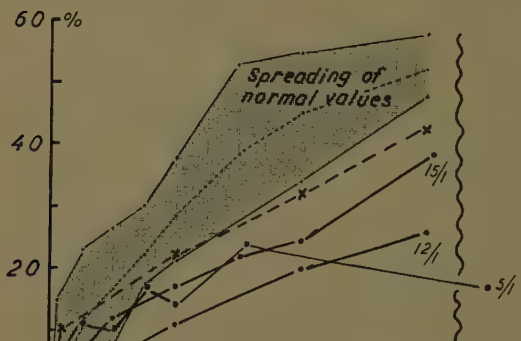

Fig. 1. The migration of P^{32} into corpuscles in a case of PNH 3 times before splenectomy and once after splenectomy.

• ——— • before splenectomy × - - - × after splenectomy
..... average values in 5 normal persons
:::::::::::: spreading of normal values.

An investigation of the intracorpuscular phosphate turnover would therefore be of interest.

THE PHOSPHATE TURNOVER IN THE RED CELLS

Inorganic phosphate entering the corpuscles is rapidly converted to organically bound phosphate (Eisenman, Ott, Smith & Winkler 1940, Hahn & Hevesy 1942, Hevesy & Aten 1939, Hevesy & Hahn 1938). Red corpuscles have an anaerobic glycolysis, in many ways resembling that of muscles and nerve tissues. The usual intermediates, ADP + hexose esters, are found and also 2,3-diphosphoglyceric acid, a compound specific to the red corpuscles. In muscles and other tissues the breakdown passes through 1,5-diphosphoglyceric acid. Dische *et al.* claim that 2,3-diphosphoglyceric acid takes part in the regulation of the acid- equilibrium within the red cells of mam — in the same way as phytinphosphoric does in birds.

Inorganic radioactive phosphate whi added to whole blood will penetrate membrane and will, in the corpuscles, part in the glycolysis. The phosph containing intermediates in the glyc will thus be marked with radiophosph In the present work these compounds chemically determined together with contents of P^{32}. In this way it was pos to follow up the phosphate transfer i red cells.

Methods

Blood was drawn and incubated as desc above. A protein-free trichloracetic acid fi was made from the blood. The following minations were made on the filtrate:

1. Orthophosphate and the radioactivity fraction. Phosphate was calculated in γ/m radioactivity in counts/min./ml.

2. 10 min. hydrolysis N/1 HCl on a b water bath. According to Bomskow (1932) fraction contains pyrophosphate split off ATP.

3. 180 min. hydrolysis in N/1 HCl unde same conditions as the previous fraction. increase in phosphate and radioactivity from 180 minutes is due to the splitting of hexosedi phoric acids.

4. Total phosphorus hydrolyzed by bu with sulfuric and perchloric acids.

Phosphate and radioactivity were deter after Ernster, Zetterström & Lindberg (1950) use the principle of Beerenblum and Chain: titative migration of phosphomolybdenic acid a water phase to an isobutanol-benzene phase aliquote was taken for the determination of activity. In another aliquot the phosphomoly acid was reduced by stannous chloride an coeroulumolybdate formed was determined electrophotometer. The rate of the phosphate

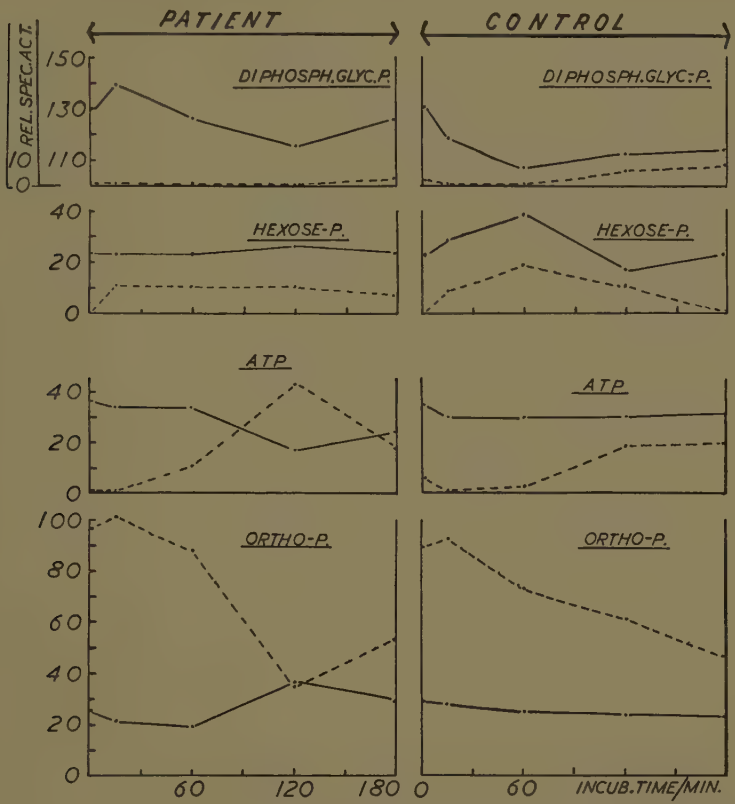

Fig. 2. Rel. sp. act. and phosphate content of various phosphate esters.
• ——— • phosphate content P/ml - - - - rel. sp. act.

is expressed as the specific activity *i. e.* the nt:

$$\frac{\text{Radioactivity}}{\text{Phosphate content}} = \frac{\text{imp./min./ml}}{\gamma\ \text{P/ml}}$$

ly intermediates in the glycolysis are rapidly d with P^{32}. ATP is one of the compounds is rapidly and intensely marked with P^{32}. ther steps in the glycolysis follow later. The te value of the specific activity depends of not only on the rate in turnover, but also on nount of P^{32} which is added to the sample. ler to compare different experiments with g concentrations of P^{32}, the specific activity orthophosphate at the time 0 is fixed to r cent. The activity of all fractions in then sed as per cent of the activity of this ortho-phosphate. This is called the relative specific activity of the compound (rel. sp. act.). Rel. sp. act. is directly comparable in different experiments.

The results are shown in Fig. 2.

Orthophosphate

During the first hour of the experiment a slight decrease in inorganic phosphorus was found both in the patient and control. The decrease continued at least 3 hours in the control. In the patient, however, 17 γ P was liberated between 60—120 min. The rise in inorganic phosphate could be accounted for by a fall in ATP of 17 γ P.

The radioactivity showed a rather rapid incorporation of inorganic phosphate in organic compounds. The rel. sp. act. of inorganic phosphate decreased in the control during the whole experiment. In the patient, however, a slight increase was found from 120 min. *i. e.* an organic compound marked with P^{32} was split.

ATP

The concentration of ATP was maintained at nearly the same level during the whole experiment in the control. In the patient, however, a splitting of ATP was found after 60 min. The fall in phosphate in ATP was in good agreement with the rise in inorganic phosphate.

The phosphate turnover was more pronounced in the patient than in the control. A higher rel. sp. act. was obtained and the incorporation of P^{32} started earlier.

The other fractions

Hexosediphosphoric acid and 2,3-diphosphoglyceric acid showed minor variations. Of some interest was the initial increase in 2,3-diphosphoglyceric acid in the patient. A change in the acid-base equilibrium within the red cell probably takes place. Later a decrease was found both in the patient and the control. After 120 min. a rise was again found in the patient. In the control the rise started again at 60 min. and was less pronounced than in the patient. It will be seen that 2,3-diphosphoglyceric acid has a slow phosphate turnover. The compound is probably labeled by phosphate transfer from an organic ester. A high specific activity could not be expected before the other organic compounds are labeled with P^{32}. After 60 min. a slight rise was found i specific activity of 2,3-diphosphogl acid. At this time the resynthesis o diphosphoglyceric acid starts.

DISCUSSION AND CONCLUSION

In PNH a retarded penetration o through the erythrocyte membrane found. This might be due to propert the membrane, but it could also explained by the metabolic processes the corpuscle.

Anaerobic glycolysis takes place i corpuscles in many ways resembling th muscles and nervous tissues. Phosph containing intermediates are found.

In vitro experiments on blood incu at 37° C in P^{32}-containing medium sh increased turnover of the labile phospha ATP in the patient. A more pronou splitting of ATP with liberation of phate was found in the patient. The ch were most pronounced between 60 an min., which is at the same time tha difference in penetration rate began. possible that the splitting of ATP wi increase in inorganic phosphate withi corpuscle counteracts the penetration organic phosphate through the memb In this case the disturbances in the bolic process might be responsible fo low penetration of the phosphate ion th the membrane.

SUMMARY

In PNH a decreased penetration of phorus through the erythrocyte mem was found by incubating blood at 37° P^{32}-containing medium. Further, differ in the phosphate turnover and stabili

within the red cells were found. ATP
ed a rapid phosphate turnover, but the
ity of the compound was disturbed.
e decrease in permeability and the dif-
ces in the metabolic processes were
d at the same time. It is therefore
ble that the change in the metabolic
is responsible for the slow penetration
e phosphate ion.

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CONDUCTIVIMETRIC DETERMINATION OF TOTAL CATIONS IN SERUM

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In 1898 Burgarszky & Tangl found that measurements of the capacity of serum to conduct electric current could be used to determine the total concentration of cations in serum.

More recently Sunderman (1945) and Holm-Jensen (1947) have used conductivity measurements to determine total cations and these authors found satisfactory agreement with other methods. By determining total cations both conductivimetrically and according to Hald's method (1933), Sunderman found an average deviation of 1.5 per cent between the two methods. Holm-Jensen has compared the results of conductivimetric total cation determination with Key's electrodialytic method (1936) and found a maximum deviation of ± 3 per cent between the two. Holm-Jensen made his comparative investigations in sera from healthy individuals, while Sunderman also examined sera from patients although no diagnoses are given.

Conductivimetry would thus appear to be a simple, rapid and accurate method for the determination of total cations. The modification described by Holm-Jensen is particularly well adapted to routine clinical use. Since determination of total cations in serum is of great clinical importance, such a simple method as the conductivimetric deserves attention.

We have performed a number of d minations of total cations *ad modum* H Jensen in sera from normal persons and some patients. The contents of the indiv cations were also determined in the sera. The results obtained by condu metry were compared with the result tained by adding the concentrations o individual cations. This provided a basi evaluating the applicability of the condu metric method for total cation determin in normal sera as well as in sera showed deviations from the normal distribution and normal protein conte

METHODS

Determination of conductivity was perf with a slight modification *ad modum* Holm- (1947). In this method the ratio of the condu of diluted serum and the conductivity of a NaCl solution is measured by means of a W stones bridge. Holm-Jensen uses a NaCl so of 150 milliequivalents/l as standard basis o parison and dilutes it the same number of as the serum, from 10 to 100. The present a have employed a total cation standard wit m eq/l consisting of NaCl 142 m eq/l, KCl 5 CaCl$_2$ 5 m eq/l and MgCl$_2$ 3 m eq/l. The a have diluted both sera and total cation st 10 times with distilled water before mea conductivity.

n determining total cations by conductivi-
t must be taken into consideration that the
e of proteins decreases the conductivity
ist 1895, Burgarszky & Tangl 1898, Gram
en 1923). It is necessary to make a correc-
or the decrease in conductivity caused by
um proteins, and in this investigation a cor-
given by Gram & Cullen (1923) has
sed.

m eq total cations/l =

$$\left[\frac{C_s}{C_{st}} + \frac{2.2 \times \text{g per cent total protein}}{100}\right] \times 155$$

= conductivity in serum diluted 1/10.
= conductivity in total cation standard
5 m eq/l) diluted 1/10.

ermination of individual cations. The con-
ion of sodium and potassium in serum was
ined by flame photometry in a Baird Asso-
Flame Photometer according to a method
d by White (1952). The calcium concen-
was determined according to Clark & Col-
nodification (1925) of Kramer & Tisdal's
l. The concentration of magnesium was not
ined by analysis, but in all the following
tion it is assumed to comprise 3 m eq/l. The
f the concentrations of Na, K, Ca and Mg,
sed in m eq/l and rounded to the nearest
number, is in the following called the real
ations. The accuracy with which this figure
determined is assumed to be around ± 2 m
The concentration of albumin and globulin
etermined by the micro-Kjeldahl method, the
ns being precipitated with sodium sulfate
1921).

RESULTS

e results of the investigation in normal
re presented in Table I.
e conductivimetric method for deter-
g total cations yields results which
good agreement with the real total
s in the 33 normal sera examined.
one exception the deviation from the

Table I. *Total cations in sera from 33 healthy individuals determined conductivimetrically and calculated by adding the concentrations of the individual cations. (Magnesium was not determined by analysis but was assumed to comprise 3 m eq/l.) The total cations are expressed in m eq/l.*

	Na	K	Ca	Real total cations	Total cations conductivimetrically determined	Difference between conductivimetrically determined total cations and real total cations
G.S.	146	4.4	5.0	158	153	—5
H.T.	138	5.0	5.3	152	155	+3
E.S.	140	4.3	5.3	153	153	0
B.S.	140	4.6	5.3	153	156	+3
O.F.	141	4.6	5.4	154	158	+4
A.G.	144	5.6	5.5	158	160	+2
A.B.	142	4.5	5.2	155	155	0
S.N.	142	4.8	5.0	155	153	—2
A.V.	144	4.5	5.1	157	156	—1
A.F.	142	4.3	5.0	154	153	—1
A.L.	142	4.3	5.0	154	151	—3
R.T.	143	4.2	5.0	155	152	—3
A.A.	142	4.5	5.7	155	152	—3
I.H.	144	4.2	5.0	156	152	—4
R.R.	144	3.9	5.3	156	153	—3
J.K.	139	5.1	5.0	152	151	—1
O.S.	141	5.1	5.0	154	151	—3
K.W.	142	4.7	5.1	155	153	—2
S.W.	141	5.1	5.1	154	151	—3
A.O.	144	5.2	5.2	157	153	—4
L.B.	142	5.1	5.1	155	154	—1
M.M.	141	5.1	5.1	154	156	+2
E.G.	141	5.0	5.0	154	153	—1
H.J.	141	5.2	5.2	154	153	—1
M.B.	139	4.6	4.6	151	154	+3
H.B.	139	4.7	4.7	151	154	+3
P.A.	139	3.9	3.9	151	154	+3
O.W.	141	4.9	4.9	153	154	+1
A.W.	143	4.5	4.5	155	155	0
K.H.	140	4.5	4.5	152	154	+2
I.J.	142	4.6	4.6	154	153	—1
R.A.	142	5.0	5.2	155	155	0
A.I.	143	4.6	5.1	156	153	—3

real total cations was whithin the limits ± 4 m eq/l. In one case the deviation was

5 m eq/l. As regards normal sera there is good agreement between the present results and those obtained by Sunderman and by Holm-Jensen.

We have also examined sera from a total of 42 patients representing an unselected group of blood samples received at the laboratory. For 15 of the patients the difference between the real and the conductivimetrically determined total cations lay within the limits ± 4 m eq/l. In the other 27 patients, however, the deviations were greater. The results of the investigations on these 27 patients are presented in Table II.

In an attempt to explain why the conductivimetric method gives less satisfactory results in some cases, we have recorded the following:

The capacity of an electrolyte solution to conduct electric current is dependent both on the total concentration of electrolytes and on which electrolytes it contains.

Conductibility increases with increasing concentration, but there is not complete proportionality between concentration and conductibility, as conductibility increases somewhat less than directly proportional to the increase in electrotlyte concentration. The conductivimetric method for total cation determination is based on the assumption that there is proportionality between conductibility and total electrolyte concentration. An error will thus be introduced when the method is employed on sera with extremely high or extremely low total cations. To ascertain the significance of this lack of proportionality in conductivimetric total cation determination, the authors have investigated the relationship between concentration and conductibility for a series of solutions of NaCl. The results are presented in Table III.

Table II. *Total cations in 27 pathol sera determined conductivimetrically calculated by adding the concentrati the individual cations. Conc. of cati in m eq/l.*

	Na	K	Ca	Real total cations	Total cations conductivimetrically determined
♂L.K. 66 years Vit. cordis	143	5.5	4.9	156	148
♀G.E. 67 years Vit. cordis	141	4.5	5.1	154	148
♀M.S. 65 years Vit. cordis	146	4.8	5.2	159	151
♀M.Ø. 71 years Vit. cordis	143	5.0	5.4	156	148
♀I.O. 60 years Vit. cordis	137	4.7	4.2	149	143
Å.S. 67 years Vit. cordis	143	3.3	4.2	154	141
♂K.W. 69 years Vit. cordis	138	5.9	4.7	152	146
♀B.H. 64 years Vit. cordis	140	5.3	4.9	153	148
♂O.S. 53 years Vit. cordis	137	3.9	5.2	149	139
♀B.S. 60 years Pneumonia	143	5.7	5.1	157	150
♂T.G. 56 years Pneumonia	134	4.5	4.4	146	139
♂H.J. 53 years Asthma bronchiale	141	5.5	4.6	154	144
♂G.V. 48 years Pneumonia	140	5.5	5.3	154	142
♀O.F. 68 years Haemorrhagia cerebri	143	5.0	5.0	156	149
♂J.K. 66 years Uremia	144	4.9	4.8	157	149
♂H.G. 59 years	138	6.6	5.1	153	145
♀D.N. 66 years Cysto-pyelitis	139	4.9	5.0	152	147

le II. (Cont.)

	Na	K	Ca	Real total cations	Total cations conductivimetrically determined	Difference between conductivimetrically determined total cations and real total cations
25 years titis acc.	141	4.5	4.9	153	144	— 9
49 years titis chron.	142	4.3	4.7	154	142	—12
58 years osis itis	134	4.0	4.0	145	154	+ 9
65 years er creatis	135	2.5	4.2	145	133	—12
51 years sis pylori	137	2.4	4.9	147	138	— 9
47 years sis pylori	138	2.8	5.6	149	135	—14
57 years omatosis	125	4.4	4.7	137	168	+31
40 years omatosis	131	5.2	4.7	144	163	+19
47 years omatosis	135	4.5	4.7	147	166	+19
50 years omatosis	136	4.5	4.9	148	163	+15

e results show that conductivimetrically nined total cations will give as much m eq/l too high values at extremely ation concentrations and up to 1.5 m oo low values when total cations are nely high.

s circumstance alone, however, cannot n the disagreement found in a number tohological sera between conductivi- ally determined and real total cations. various electrolytes, however, have nt specific conductivity and a change

Table III. *The relationship between concentration and conductibility for solutions of sodium chloride. Conc. in m eq/l.*

Real concentration.	100	120	140	180
The concentration conductivimetrically determined. Standard: NaCl-solution of 140 m eq/l.	102.5	122	140	178.5

in their mutual proportions will thus influence conductivity.

The potassium-sodium relationship. The potassium ion has a conductivity which is about 17 per cent higher than that of the sodium ion. Thus, if the concentration of potassium is increased by 3 m eq/l, the increase in conductivity will be the same as if the sodium concentration had been increased by 3.5 m eq/l. As we always measure the conductivity of serum against a standard consisting largely of NaCl, an increase in the potassium concentration of 3 m eq/l will correspond to an increase of 3.5 m eq/l in the total cations. As the potassium concentration in serum, which is normally 4—5 m eq/l, may vary under pathological conditions between 1 and 10 m eq/l, pathological potassium values will lead to error of up to — 0.5 and + 1 m eq/l total cations per l.

Calcium. Serum calcium is present only in part in ionized condition. As it is only the ionized calcium which conducts electric current, the degree of ionization of the calcium will influence the conductivity of the serum. All of the factors which influence the ionization of the calcium salts (pH of the blood, concentration of bicarbonate and phosphate as well as the presence of organic acids which can form a calcium complex) will thus be able to influence the conductivity of

Table IV. *The influence of the citrate ions on the conductibility of solutions of electrolytes containing calcium ions. Conc. in m eq/l.*

Conc. of NaCl	155	155	150
—»— Na-citrate	5	0	5
—»— $CaCl_2$	0	5	5
Real total cations	160	160	160
Total cations conductivimetrically determined	160	160	155

the serum. Table IV represents a model experiment which shows that the addition of citrate binds the calcium ions in such a manner that they no longer have any conductivity.

In pathological sera where the calcium may be more or less ionized than in normal sera, this circumstance may be a source of discrepancy between conductivimetrically determined and real total cations.

The chloride-bicarbonate relationship. As the various anions have different conductibility, any changes in their mutual relationships will also influence the conductibility of the serum.

The conductibility of a solution of sodium bicarbonate is only 77 per cent of the conductibility of an equimolar solution of NaCl. Table V shows a model experiment which illustrates how the conductibility in solutions containing NaCl and $NaHCO_3$ varies with the ratio of the two components.

The concentration of sodium bicarbonate in serum, normally about 25 m eq/l, may, under pathological conditions, vary from 5 to 60 m eq/l. Pathological shifts in the concentration of bicarbonate may thus lead to

Table V. *Total cations conductivim cally determined in solutions containing NaCl and $NaHCO_3$, with varying rat the two components. Conc. in m eq*

Concentration of NaCl	160	140	100	80
Concentration of $NaHCO_3$	0	20	60	80
Sum	160	160	160	160
Total cations conductivimetrically determined. Standard: NaCl-solution 160 m eq/l	160	155	146	142

error up to + 5 and — 8 m eq/l total c in the conductivimetric method.

Proteins. The mutual relationship bet the various serum protein fractions may considerably under pathological condit It is therefore of interest to asce whether the various protein fractions de conductibility to the same extent.

Normal human albumin was prepare fractionated alcohol precipitation at 0°. alcohol was removed by freeze-drying the freezedried albumin was dissolve NaCl solution. Electrophoretic examin of this solution proved it to be pract free of globulins. The solution's conte cations and albumin were determined a conductibility was measured in relati a pure NaCl solution. The results are sented in Table VI.

It appears from this experiment th albumins have a marked depressive eff conductibility. Per gram per cent of min, this decrease in conductibility is than 3 per cent.

Commercial human gamma globuli dissolved in NaCl solution. Electroph

le VI. *The influence of albumin on ctibility. Comparison between the ctibility in albumin-electrolyte solution the conductibility in NaCl solution. Conc. of proteins in g per cent. Conc. of cations in m eq/l.*

in	Na	K	Ca	Real Total cations	Total cations conductivimetrically determined. Standard: NaCl solution 100 m eq/l. No correction for the presence of protein has been added
105	1.4	1.9		108	98
100	0	0		100	100

Table VII. *The influence of γ-globulin on conductibility. Comparison between the conductibility in a γ-globulin — NaCl solution and the conductibility in NaCl solution. Conc. of proteins in g per cent. Conc. of cations in m eq/l.*

γ-globulin	Na	K	Ca	Real total cations	Total cations conductivimetrically determined. Standard: NaCl solution 140 m eq/l. No correction for the presence of protein has been added.
3.6	131	0	0	131	128
0	140	0	0	140	140

nation of this solution demonstrated t contained no other protein fractions. rotein and cation contents of the solu- vere determined and its conductibility ired in relation to a pure NaCl solution. esult is presented in Table VII which that the gamma globulins have little nce on conductibility. Calculated per per cent gamma globulin the decrease nductibility is less than 1 per cent.

have also determined conductibility in me serum before and after the removal bulins, and in this manner have also able to study the influence of the protein fractions on conductibility. The globulins were removed by adding ice-cold alcohol to the serum until the alcohol concentration reached 40 volume per cent. The precipitated globulins were removed by centrifugation at 0°. In order to remove the alcohol, the supernatant was freeze-dried and then dissolved in so much distilled water that the original volume was recovered. The content of cations and proteins as well as the conductibility were determined both in the original serum and in the »globulin-free« serum. The results are presented in Table VIII. It is

Table VIII. *The conductibility of a sample of serum before and after the removal of the globulins. Conc. of proteins in g per cent. Conc. of cations in m eq/l.*

	Total Proteins	Alb.	Glob.	Na	K	Ca	Real total cations	«Total cations» conductivimetrically determined without any correction for the presence of proteins
l serum rum after ving the ulins	6.6	4.4	2.2	142	6.8	5.0	157	136
	4.1	3.8	0.3	142	6.8	1.9	154	136

Table IX. *The content of "total cations" conductivimetrically determined in sera without adding the correction for the presence of proteins. Sera and the total cation standard of comparison diluted to the same degree. Total cation standard: 155 m eq/l. Conc. in m eq/l.*

		«Total cations» conductivimetrically determined	Difference between the real total cations and the «total cations» conductivimetrically determined
Normal serum Real total cations: 154 m eq/l Total proteins: 7.0 g per cent	undiluted	117	37
	diluted 1/5	128	26
	—»— 1/10	130	24
	—»— 1/100	133	21
Myelomatosis Real total cations: 144 m eq/l Total proteins: 10.1 g per cent	undiluted	108	36
	diluted 1/5	127	17
	—»— 1/10	129	15
	—»— 1/100	132	12

seen that conductibility is unaffected by the removal of the globulins.

These experiments show that the globulins have little influence on conductibility. Use of a constant correction factor will lead to inaccuracies when the albumin/globulin ratio deviates from the normal.

Holm-Jensen states that the influence of proteins on conductibility can be neglected when the serum is diluted as much as 100 times. As it would be a great advantage to be able to eliminate the depressive effect of the proteins on conductibility, we have investigated the relationship between conductibility in serum and in total cation standard* at the same dilutions. These experiments are represented in Table IX. We cannot agree that the proteins have no influence on conductibility when the serum is diluted times.

On the basis of these experiments we investigated to what extent pathological centrations of bicarbonate can explain discrepancies between the real and the ductivimetrically determined total cat By correcting conductibility to what it w have been at a normal concentration of b bonate, much better agreement was fou six patients between the real and the ductivimetrically determined total ca The correction was based on the follo facts: The conductibility of a solutio sodium bicarbonate is only 77 per ce the conductibility of an equimolar soluti sodium chlorides. The total cation stan of comparison consists of chlorides 10 m eq/l increase of the concentrati bicarbonate thus leads to an increase o conductivimetrically determined total ca

* Described on page 126.

le X. *Total cations conductivimetri- determined in sera with pathological ntration of bicarbonate. The results are orrected to what they would have been a normal bicarbonate value. Conc. in m eq/l.*

	Conc. of chloride	Conc. of bicarbonate	Real total cations	Total cation conductivimetrically determined	Corrected results
M. rrhosis hepatis	120	17	145	154	152
S. . cordis	89	38	154	141	144
G. eumonia	87	28	146	139	140
N. ncer pancreatis	92	33	145	133	135
G. nosis pylori	49	57	149	135	144
nosis pylori	52	63	147	138	147
er recovery	90	27	149	149	150

m eq/l only. A correction of 2.3 m eq/l be added. The results are represented ble X.

six patients with pathological serum n values the disagreement found be- the real and the conductivimetrically nined total cations, could be explained extremely high globulin content. By ating the conductivimetric total cations ese sera with a correction for the ins only — disregarding the globulins ly — much better agreement was found. er details concerning these sera will be in Table XI.

SUMMARY

We have demonstrated some factors which may contribute to an explanation of the discrepancies found in pathological sera between the results of the conductivimetric method for determination of total cations and ordinary chemical analysis. The most important of these is the fact that the specific conductibility of the many ions present in serum differs, and therefore alterations in the mutual relationships between the concen-

Table XI. *Total cations conductivimetrically determined in sera with pathological serum protein values. The results are given both with correction for total proteins and with correction for the albumins only.*

	Conc. of albumin	Conc. of globulins	Conc. of totalproteins	Real total cation	Total cations conductivimetrically determined with correction for totalproteins	With correction for albumin only
O.M. Cirrhosis hepatis	3.6	3.8	7.4	145	154	143
A.D. Cirrhosis hepatis	2.5	6.9	9.4	146	155	136
O.H. Myelomatosis	2.6	12.0	14.6	137	168	135
J.H. Myelomatosis	2.8	7.3	10.1	144	163	138
O.R. Myelomatosis	3.1	6.6	9.7	147	166	143
H.T. Myelomatosis	4.1	6.5	10.6	148	163	145

trations of these various ions will necessarily lead to inaccurate results with the conductivimetric method. This is true of shifts in the ratio between cations, but even more so when the ratios of the anions are altered. Most significant are alterations in the bicarbonate/chloride ratio and in the ratio between the albumin and globulin values. When proper consideration is taken of the specific conductibility of the anions, it is possible in many cases to obtain better agreement between total cations determined conductivimetrically and by chemical analysis.

Another factor which may have a disturbing influence is a change in the degree of ionization of calcium, perhaps also of magnesium under pathological conditions. Furthermore, it is important to be aware of the fact that there is not complete proportionality between total cations and conductibility, as assumed in the conductivimetric total cation method.

We have made conductibility determinations in a serum dilution of 1/10 as also Holm-Jensen has done. He claims that the depressive effect of the proteins on conductibility will be negligible in a dilution of 1/100. We have not been able to confirm this. The depressive influence on conductibility was less at dilution 1/100 than in a dilution of 1/10, but it was still considerable.

CONCLUSION

The conductivimetric method for determination of total cations in serum *ad modum* Holm-Jensen gives satisfactory results in normal sera. In pathological sera the results are unreliable because conductibility is dependent not only on the total electrolyte concentration but is also influenced consider by the mutual relationships between the centrations of the various serum electrol In particular, deviations from the no chloride/bicarbonate ratio and the no albumin/globulin ratio will disturb the r The method can be improved somewha making corrections for such patholo values, but at the same time it becom complicated that it no longer has any tical significance.

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PLASMA PROTEIN CHANGES AFTER ACUTE MYOCARDIAL INFARCTION

ALPHA$_2$- GLOBULIN AND FIBRINOGEN

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ring recent years attemps have been by numerous investigators to elucidate hanges occurring in the protein frac- of the blood in cases of myocardial ction. Several investigators have also pted to determine the relation between changes an the increase in the sedimen- rate which is observed during the ess of the disease.

ith the aid of electrophoresis, Donzelot, fman & de Mende (1949) found in cases of myocardial infarction that the nt of β-globulin increased during the two days and that the amount of bulin began to increase on the third day. g paper they finally attained a level that —4 times the first value. Hauss & Leist 3) employed Antweiler's method in the sis of the proteins in the sera of 33 nts with mycardial infarction. The albu- underwent the usual decrease, the α- lin increased in the initial stages and the bulin in the later stages. Using paper ophoresis, Gräsbeck (1953) studied the ges in serum proteins following myo- al infarction (nine cases); his results similar to those obtained by Donzelot

rticular attention has been paid to the tion in the fibrinogen following myocardial infarction and its effect on the sedimentation rate. Different methods have been used to determine the fibrinogen, but the common finding has been that the amount of fibrinogen strongly influences the sedimentation rate. This effect becomes evident already at the end of the first week after the onset of the infarction (Meyers, 1948; Gilchrist & Tulloch, 1952 and recently Losner, Volk & Wilensky, 1954).

The serum mucoprotein has also been observed to undergo a marked increase after myocardial infarction (Simkin *et al.*, 1949; Taipale, 1954).

The methods used in these studies differ in type and different investigators have been primarily interested in different protein fractions. In fact, however, these factors and their changes are far from independent of each other.

In the present study, which is a part of a more extensive analysis of the changes which occur in the plasma protein fractions, including the mucoprotein, after myocardial infarction, attention has been primarily paid to the variations in the α_2-globulin and fibrinogen fractions and to the influence of these changes on the development of the erythrocyte sedimentation rate.

MATERIAL AND METHODS

The present series comprises 49 clinically proved cases of acute myocardinal infarction and 8 cases of coronary failure in which the occurrence of infarction was doubtful. Blood samples for the electrophoretic analysis of the plasma were taken, when possible, on the day the infarction occurred, on second, third and fourth days, on the sixth day, and subsequently at weekly intervals as long as the patient remained in hospital. One sample was again taken during convalescence approximately one month after discharge. All the patients in question received anticoagulant treatment (Heparin, Phenylindandione), which according to general criteria can be considered adequate, from the day of admission.

The data presented below are based on 300 electrophoretic analyses of plasma. In these analyses Antweiler's micromethod has been employed, the buffer solution used being Michaelis' oxalate buffer (pH 9.1). The samples were taken int tubes containing desiccated potassium oxalate analyses were performed with the microel phoresis apparatus developed by Antweiler kamp). The total protein has been determined at least two methods, one a semi-micro-Kje and the other, one of the following: van S falling drop in copper sulphate, Greenberg's metric method, or Antweiler's method.

For the evaluation of the relationship be the sedimentation rate (Westergren) and th globulin content and that between the rate an fibrinogen content, correlation coefficients (r) been calculated according to Bravais-Pearso

Table I presents the average values an ranges for the various fractions obtained control group of the present study. The figu brackets give the average values for plasm healthy subjects according to Schuler.

Table I. *Normal electrophoretic plasma protein values for 19 healthy persons (a from 22 to 65 years) determined as double analyses. Antweiler's (Boskamp) apparat Michaelis' oxalate buffer (pH 9.1); 16—18 min., temp. about + 12° C. The figures brackets give the results obtained by Schuler using the same method of analysis.*

Per cent of total protein	Alb. + α_1	α_2	β	φ	γ
Mean	60.5 (59.5)	6.9 (6.0)	9.2 (10.5)	6.0 (7.0)	17.5 (17.0)
Range	54—66	4.5—8.9	7.8—10.6	3.0—8.0	11.4—22.6

RESULTS

The α_2-globulin fraction is the one in which the first changes are observed. An increase in this fraction is observed already during the first three days after the onset of infarction. The correlation between the α_2-globulin content and the sedimentation rate and that between the fibrinogen content and the rate during the first three days is shown in Fig. 1. Although the mean part of the fibrinogen values remain within the n limits, there appears to be some corre between the φ values and the sedimen rate, since the correlation coefficien has a relatively high value, + 0.43 ± This is evidently due to the number of high values, up to 15 per cent, foun fibrinogen at this stage. The positive relation coefficients indicate that protein fractions influence the sedimen

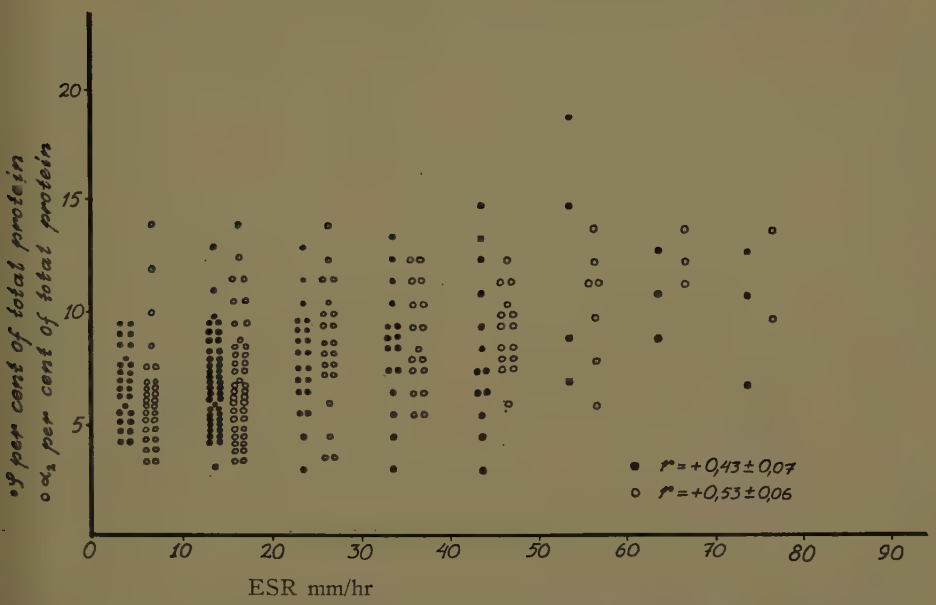

Fig. 1. Samples taken on the first three days after the onset of the infarction. (129 analyses.) $r\alpha_2 = +0.53 \pm 0.06$; $r\varphi = +0.43 \pm 0.07$.

lready during the first three days after set of the infarction.

fairly strong correlation ($r = +0.58$ 8) between the α_2-globulin and the entation rate is observed for the l from the 4th to the 9th day. The ogen content has undergone a general e which parallels the increase in the entation rate; as a result, the correla- s quite strong ($r = +0.78 \pm 0.05$). effect of fibrinogen content on the entation rate is known to be marked. orrelation found is in accordance with esults of Meyers and of Gilchrist & h who found that the fibrinogen t attain its maximum value one week the onset of myocardial infarction. r, Volk & Wilensky, however, have that the fibrinogen content may frequently have a high value in the initial stages of the disease although the sedimentation rate has not increased, whereas in some cases the sedimentation rate may remain high although the fibrinogen content has become normal. Exceptions of this kind are also noted in the present material.

In the samples taken from the tenth to the 23rd day, a correlation between the α_2-globulin content and the sedimentation rate is still evident ($r = +0.45 \pm 0.04$). The fibrinogen content, however, still remains above the normal level at this stage and show a significant positive correlation with the sedimentation rate ($r = +0.53 \pm 0.09$).

The number of samples taken at later stages or during convalescence were so few in number that no statistical calculation of correlation coefficients can be made.

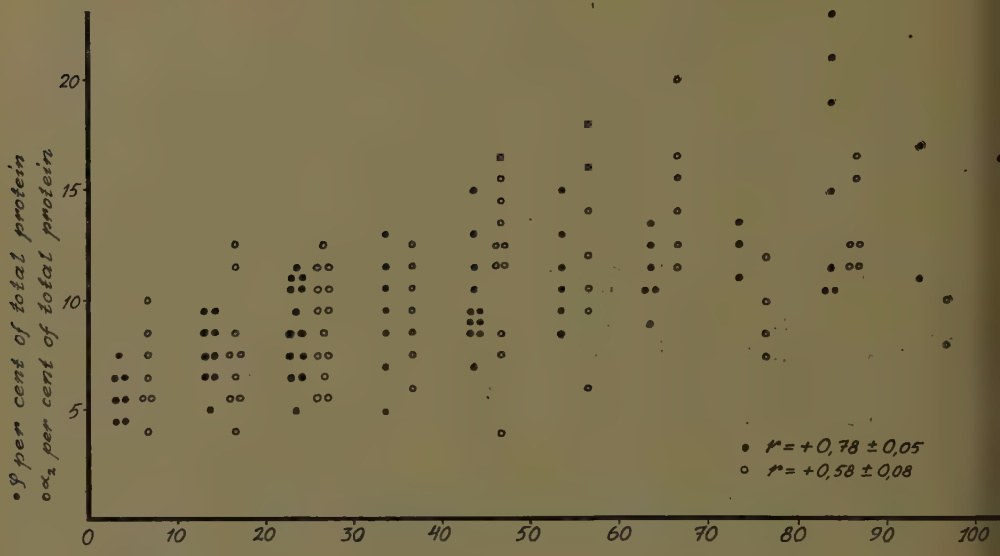

Fig. 2. Samples taken on the 4th to 9th days after onset of the infarction. (74 analyses). $r\alpha_2 = +0.58 \pm 0.08$; $r\varphi = +0.78 \pm 0.05$.

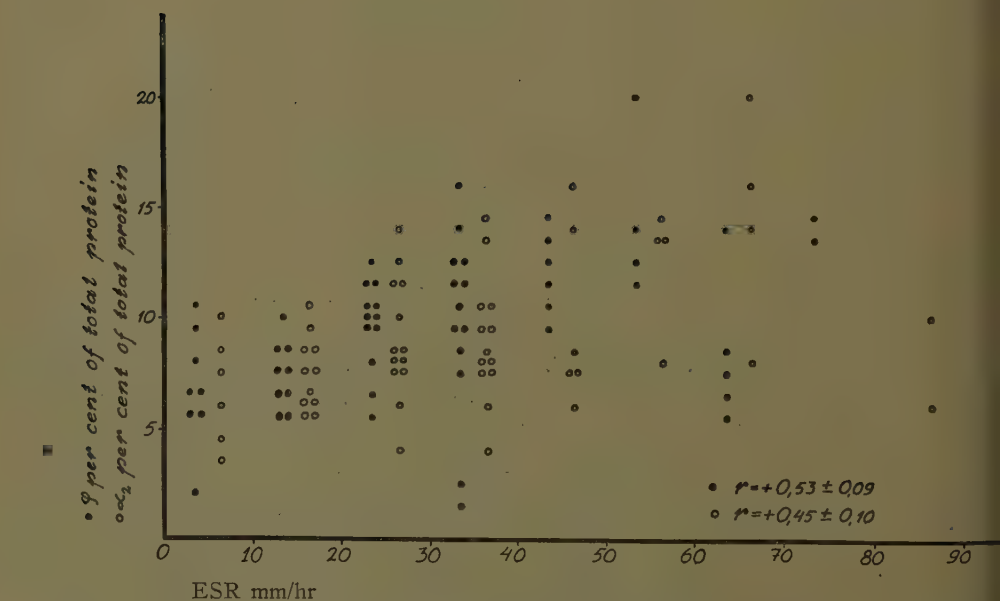

Fig. 3. Samples taken on the 10th to 23rd days after onset of the infarction. (59 analyses). $r\alpha_2 = +0.45 \pm 0.10$; $r\varphi = +0.53 \pm 0.09$.

eas the sedimentation rate remained elevated in these subjects, values were ed for certain protein fractions (α, β, which are higher than normal, or at he ratio of albumin to globulin is less ormal.

COMMENT

ing to the fact that the methods used dy the changes in the protein fractions blood which are associated with myo- l infarction differ greatly, it is ult to compare the results of different igators. Previously the electrophoretic es have been made on serum samples, as in the present study the plasma has analysed. The electrophoretic analysis ch more difficult in the latter case. trong *et al.* (1947) state that small its of β- and γ-globulins are carried with the fibrinogen and *vice versa.* greatest difficulty encountered in the t method is that, for some reason or the fibrinogen does not separate etely from these globulin fractions and consequence in many cases only one ned wave is obtained. Especially the ogen and β-globulin fraction move in sequence.

SUMMARY

ctrophoretic analyses using Antweiler's method have been conducted to de- e the changes in the proteins of the a in 49 clinically proved cases of acute rdial infarction and in 8 cases of ary failure in which the occurrence of tion was doubtful.

he present paper only the relationship en the sedimentation rate and the α_2- in and fibrinogen fractions at various stages of acute myocardial infarction is considered. Both fractions were found to have a clear influence on the variation of the sedimentation rate within the first three days after the onset of the infarction. From the 4th to the 9th day, the effect of the fibrinogen seems to be more prominent, but later no marked difference is noted in the effect of these two factors.

Since the increase in the sedimentation rate which follows the onset of myocardial infarction is evidently affected by the simultaneous operation of many factors and several protein fractions are also involved which do not vary independently of each other, the present results must be considered preliminary. Work in progress on the changes in other fractions of the plasma is expected to elucidate the relative significance of the various factors influencing the changes in the sedimentation rate.

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THE SERUM MUCOPROTEIN CONTENT AND ACUTE MYOCARDIAL INFARCTION

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ıte myocardial infarction effects marked ions in the serum albumin and globulin ons and in the fibrinogen content of ood plasma (Meyers, 1948; Donzelot, mann & Mende, 1949, Gilchrist & ch, 1952; Gräsbeck, 1952, Hauss & 1953; Sonnet & Sibille, 1953; Linko aris, 1955). These changes are partly nsible for the observed increase in the rocyte sedimentation rate (ESR).

atively little attention has, however, paid to the variation of the serum proteins. Mucoprotein (Mp) is a glyco- n complex which accompanies a_1-globu- nd possibly also albumin in electro- sis (Winzler & Smyth, 1948; Winzler, , Mehl & Smyth, 1948; at pH 4.5) different components may be distin- ed in the electrophoretic pattern. Muco- n is soluble in sulphosalicylic acid, is agulated by heat and is salted out by nium sulphate. According to Winzler, tains approximately 58 per cent pro- 15 per cent carbohydrates, 12 per cent amines and 13 per cent lipids.

e serum mucoprotein content is known crease in infectious and neoplastic es, but it decreases in cases of liver chyma damage (Winzler *et al.*, 1948; span, Tepper, Terry & Schoenbach, 1952; Greenspan, 1954). Since an increase in the mucoprotein is a characteristic feature of diseases causing tissue destruction, Simkin, Bergman & Prinzmetal (1948) carried out determinations of serum mucoprotein on patients with myocardial infarction and found that a considerable rise occurs in the Mp content in this disease. This result has been confirmed by Taipale (1954). Simkin *et al.* established that the increase in mucoprotein is observed already on the third day after the infarction and that it attains its maximal value by the sixth day on the average. Subsequently the mucoprotein value decreases and reverts to normal in the course of four weeks. From their study of a series comprising 23 cases, these authors came to the conclusion that the changes in the Mp content may be of value in the diagnosis of myocardial infarction, since the mucoprotein content is a more sensitive indicator than, for example, the ESR and the leukocyte reaction.

The purpose of the present study has been to follow the rate of variation of mucoprotein content by conducting serial determinations and to compare this variation with the changes occurring in the ESR and the plasma fibrinogen.

MATERIAL AND METHODS

The observations described below relate to 39 patients with acute myocardial infarction. The diagnoses have been based on clinical, laboratory and electrocardiographic findings. The time of onset of the infarction has been estimated with a fairly high accuracy on the basis of the information given by the patients. Blood samples were taken after the following periods: within 24 hours (immediately on admission to the hospital), and 1, 2, 3, 4, 6—7, 14, 21, and 28 days after the attack. Observations were also made on 12 other patients, whose diagnoses will be presented later when the results for this group are described.

The determination of serum mucoprotein was performed by the method developed by Winzler. The mucoprotein in the filtrate obtained after precipitation of the proteins with perchloric acid was precipitated with phosphotungstic acid, dissolved in sodium hydroxide and the color produced by adding biuret reagent was measured with a photoelectric colorimeter (Coleman Junior). The ESR was determined by the Westergren method. Plasma fibrinogen was estimated by the Antweiler microelectrophoresis method. All determinations were carried out in parallel on the same days.

The mucoprotein values for 21 healthy controls fell within the limits 35 and 90 mg per 100 ml of serum with an average value of 57.6 mg/100 ml. These results are in excellent agreement with the values reported by Winzler *et al.*, Simkin *et al.* and Greenspan.

The standard error of the difference betweeen two Mp values as determined for the controls was found to be 3.03 mg/100 ml. On this basis a difference of 10 mg/100 ml ($3 \times 3.03 = 9.1$) has been considered to represent a significant change in the mucoprotein content. The standard error of the difference between ESR values was found to be 2.8 mm/hr, 10 mm/hr being considered a significant difference.

RESULTS

It was possible to follow the variation of the mucoprotein content from the initial stages of the disease in 33 of the 39 cases. In the remaining six cases, the first mucoprotein determination was made at s a stage that this and the subsequent dete nations on these cases could not be incl in the evaluation of the development o serum mucoprotein content.

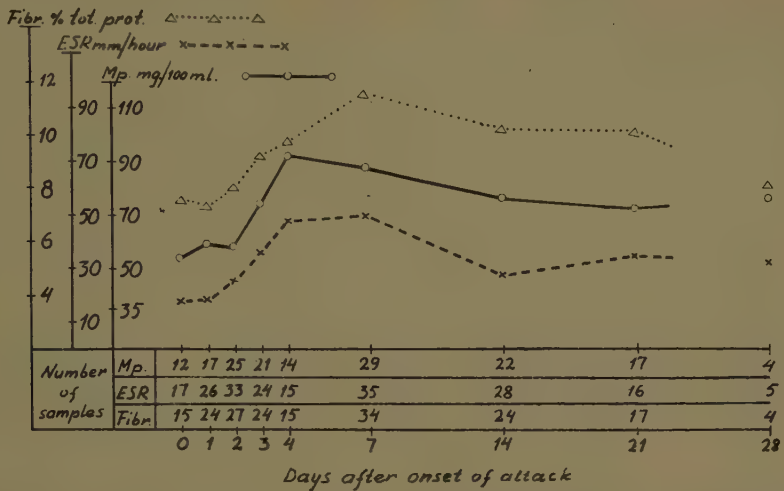
A significant increase in the Mp co ($\geqq 10$ mg/100 ml) was established 1—4 days in 25 cases, within 6—10 in 3 cases and after 14 days in one case four cases, the increase in the serum Mp so slight compared with the initial value it could not be considered significant.

The range of the initial mucoprotein v was 30.5 to 83 mg/100 ml and hence the normal limits. The changes in the content varied between 7 and 127 mg/10 the range of the maximal values being 208 mg/100 ml. Maximum values exce the normal upper limit (90 mg/100 ml) obtained in 23 of the cases.

The mucoprotein contents attained mum values in 14 cases within 1—4 in 8 cases within 7—10 days, in 5 within 14 days and in one case 28 after the attack. Seven of the patients during the first few days, and hence ma values were not obtained for these case 4 cases, no significant change in mucopr content was established.

The ESR determinations were mad the same blood samples as the mucopr determinations. Significant changes in ($\geqq 10$ mg/hr) were observed as follow 28 cases during 1—4 days and in 3 during 6—10 days. In 2 cases no signi changes were noted.

When the mucoprotein values (Mp the ESR values were compared, th lowing was observed:



Variation of serum mucoprotein (Mp), ESR and plasma fibrinogen (Fibr.) in 39 cases of acute myocardial infarction.

cant change:

Mp before one in ESR	7	cases
multaneously in both Mp and SR	10	»
ESR before one in Mp....	11	»
Mp but not in ESR	1	»
ESR but not in Mp	3	»
neither Mp nor ESR.....	1	»

plasma fibrinogen content followed riation of the ESR values fairly closely, to be expected (Fig. 1).

decrease of the mucoprotein values normal level required widely varying s of time, from four days to over days. In 15 cases that were followed longer period, the values decreased initial level within four weeks in and within 4—8 weeks in three. In ther cases, this decrease required 5, weeks.

results obtained for the group of 12 were the following: In 4 cases of suspected myocardial infarction, but without electrocardiographic changes, significant hcanges in Mp, ESR and fibrinogen were observed in 3 cases. In 4 cases with angina, without verified infarction, significant increase in Mp, but none in ESR and fibrinogen, was observed in 2 cases. In 3 cases with angina associated with various infections, significant increase in ESR and fibrinogen was noted in 3 cases, and significant increase in Mp in two cases. In one further case with intercostal neuralgia, no significant change was noted in any of these values.

The average mucoprotein, ESR and fibrinogen values obtained at different times after the onset of the disease were observed to vary in a parallel manner (Fig. 1).

To determine the relationship between the changes in the mucoprotein content and those shown by the ESR and fibrinogen values, correlation coefficients (r) have been calculated. The values obtained are: Mp/ESR,

Table I. *Initial and maximum values of serum mucoprotein and ESR in 12 c of acute myocardial infarction with electrocardiographic changes indicating exten anteroseptal and/or anterolateral infarction. The figures in brackets give the day onset of the infarction.*

Case No.	Mp initial	Mp max.	ESR initial	ESR max.
15	60 (3)	77 (10)	6 (3)	11 (5)
16	53 (<1)	133 (3)	9 (<1)	20 (3)
18	52 (1)	95 (3)	43 (1)	58 (2)
22	83 (1)	172 (4)	16 (<1)	55 (7)
31	52 (<1)	81 (1)	10 (<1)	44 (7)
32	52 (<1)	105 (3)	6 (<1)	80 (7)
38	33 (2)	50 (4)	14 (2)	24 (4)
44	32 (<1)	127 (7)	22 (<1)	98 (7)
50	34 (<1)	56 (14)	10 (<1)	15 (2)
52	42 (<1)	63 (3)	9 (<1)	66 (7)
54	38 (2)	53 (3)	4 (<1)	55 (3)
64	86 (2)	100 (3)	39 (2)	103 (4)

$r = 0.21 \pm 0.07$; Mp/Fibr., $r = 0.22 \pm 0.07$. The correlations are rather weak, *i. e.*, when all the values determined are taken into account, the Mp content may vary relatively independent of the ESR and fibrinogen values.

If we assume that the mucoprotein content of the serum is a measure of the extent of tissue destruction in myocardial infarction, the values obtained for the former should be compared with the changes noted in the electrocardiograms, which are the only criteria on which it has been customary to base an estimation of the extent of the infarction *intra vitam*.

Table 1 presents those of the present cases in whom the elctrocardiographic changes (in leads V 1—6) indicated extensive anteroseptal and/or antero-lateral infarctions of transmural or subtransmural type.

In all these cases, the increase in the mucoprotein value was significant, but only in six cases did the maximal Mp values exceed the accepted normal upper limit (90 mg/100 ml). The highest values measured in this study were obtained in this group, but were also cases among the others for the increase of the Mp content from initial value was relatively low, althoug electrocardiographic changes indicated e sive infarctions (Cases Nos. 15, 38, 5 54 and 64). When the mucoprotein and ESR values for these cases are pared, it is seen that they did not a vary in parallel.

In the cases where the infarctions we a lesser degree as judged from the ECG Mp values showed considerable variati

DISCUSSION

The data of the present study show the mucoprotein content of the serum rise to fairly high values (up to 208 m ml) following acute myocardial infar the relative increase from the initial being as high as 400 per cent in some These findings are very similar to tho for instance, Simkin *et al.* and Ta although the maximum levels attained generally been lower in the present s

initial values (30.5—83 mg/100 ml) within the normal limits. When the ance of these values is considered, it be noted that only those cases for it was possible to determine the muco- n content in the early stage of the e can be taken into account, since ise Mp values deviating from the nor- alues for the patients would be in-

ignificant increase in the Mp value mg/100 ml) from the initial values stablished within four days in three- s of the cases, which is in agreement reviously reported findings. Maximal rotein levels were attained within 1—4 n one-half of the cases and during the n days of the disease in three quarters cases.

time required for the mucoprotein to return to the initial level does not o be connected in any way with the t level attained nor with the relative se from the initial value. It was noted, er, that the mean mucoprotein value e series remained above the average initial values for a period of at least weeks. Only those Mp values were n calculating the mean which could attributed to other causes.

mucoprotein values followed the on of the ESR and fibrinogen values majority of the cases, the calculated tion coefficients showed, however, a low degree of dependence.

Mp content did not increase more than the ESR values in the initial of the disease. This observation does pport the conclusion of Simkin *et al.* that the mucoprotein content responds more sensitively than the ESR to tissue damage accompanying myocardial infarction. Similar results were obtained for a series of 12 patients in which the existence of myocardial infarction was not established or was doubtful: significant increases in Mp were no more frequent than corresponding changes in the ESR and fibrinogen values.

When considering the significance of the mucoprotein content, the relatively low standard error (about 2 mg/100 ml) of the method employed to determine the content should be noted. On the other hand, the normal mucoprotein values vary within fairly wide limits, between 35 and 90 mg/100 ml in the present control group. For this reason serial determinations of the mucoprotein content must be performed before a reliable appraisal can be made. It should also be noted that the Mp content, like the ESR and fibrinogen values, are not specific by nature, but may be altered also by various infections and, for instance, by thromboembolic complications, which possibility diminishes the significance of variations in the mucoprotein content in myocardial infarction.

It was not possible to find any definite relationship between the extent of the infarction as estimated from the electrocardiographic changes and the mucoprotein content. The small size of the present series does not permit reliable conclusions, but it was observed that the mucoprotein values for the cases for which the infarcted areas were largest as estimated from the changes in leads V 1—6, were the highest of those recorded.

SUMMARY

From a study of 39 cases of acute myocardial infarction and of 11 coronary patients in which myocardial infarction could not be established or was doubtful, it has been concluded that the serum mucoprotein content is no more rapid or sensitive an indicator of myocardial damage than the ESR, even when the errors in the determination of these values are taken into account.

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ANALYSIS OF PROTEIN-BOUND SERUM POLYSACCHARIDES WITH ANTHRONE REAGENT

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use of anthrone as a qualitative ydrate reagent was commenced by ood (1946), and since then it has sed for quantitative analyses of mono well as polysaccharides. Graff, pan, Lehman & Holechek (1951) ed a method to determine protein- hexose in serum with the anthrone . Shetlar (1952) and Tuller & g (1954) showed that when trypto- vas present in the reaction mixture, ydrate, tryptophan and anthrone with each other. This reaction t gave a 530 mμ absorbtion peak and ed with the reaction between ne and carbohydrate which gave a μ peak. This 630 mμ peak results in a lower value than would have obtained if the reaction between han, carbohydrate and anthrone had en place.

determine the suitability of the ne method for analysis of protein- hexose in blood serum, 32 healthy uals, predominantly men, between the f 18—35 have been investigated ng to Graff, and to a modification of ethod worked out by the author. ; the error conditioned by the han content of the serum protein has been estimated and the possibility of making a correction for this error has been indicated.

Graff's method, which utilizes the reaction heat generated upon mixing the concentrated sulphuric acid in the reagent and the test solution, gives relatively large variations in duplicate determinations. Because of this, a series of runs were made in order to standardize the time and temperature. Fig. 1 shows the absorbancy in relation to time and temperature. At 100° C the absorbancy at 630 mμ wave length attained a maximum after 10 minutes and then decreased quickly. At 90° C the maximum came somewhat later, and the absorbancy decreased slightly during the next 20 minutes. Therefore the 90° C temperature is to be preferred.

METHODS

The following modification of Graff's method has been used in experiments reported here:

Reagent

0.2 per cent anthrone (wt/vol) in concentrated sulphuric acid A. R. sp. gr. 1.84. Anthrone was synthesized according to Meyer (1932). The anthrone reagent will remain stable about 2 weeks if kept in a refrigerator at about + 4° C.

Hexose standard solution: 50 mg galactose + 50 mg mannose + 0.25 g benzoic acid were dissolved in distilled water to 100 ml by volume. Before use this solution was diluted 1:10 with distilled water.

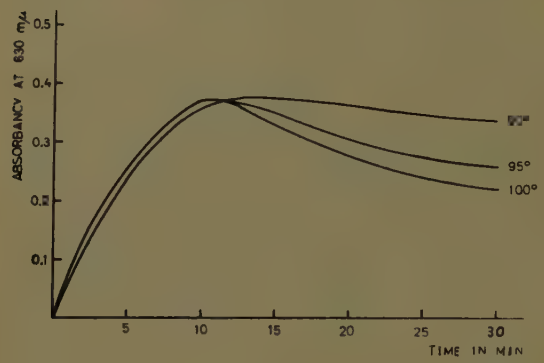


Fig. 1. Estimation of protein-bound serum hexose with anthrone reagent. Development of color as a function of time and reaction temperature.

Procedure

Serum was diluted with the double volume of 0.9 per cent NaCl. 0.4 ml of the diluted serum was added dropwise to a flat bottomed glass tube which contained 10 ml absolute ethanol. The serum polysaccharides then precipitated with the protein. After careful mixing, the solution was centrifuged at 2.000 r.p.m. for 10 minutes. Then the ethanol was decanted and the precipitate was washed once with an additional 10 ml ethanol. After centrifuging the precipitate was dissolved in 4 ml distilled water. Distilled water was used as a blank. The test samples and the anthrone reagent were cooled to + 4° C. 8 ml of the reagent were carefully layered with a pipette into the test tubes and the reagent and test solutions were mixed in an ice cold water bath. In this way the temperature during mixing could be held so low that color development was insignificant. The mixed solutions were then transferred to well stoppered glass tubes and heated in a water bath at 90° C for 14 minutes to complete the reaction. The tubes were cooled in ice water, and the absorbancy at 630 mμ was read on a Beckman B spectrophotometer against distilled water. The absorbancy for the blank should not exceed 0.02—0.03. The amount of protein-bound hexose in mg per 100 ml of serum was estimated from a standard curve. In Graff's method readings were made at 620 mμ; however, the maximum absorbancy was found at 630 mμ.

RESULTS

With Graff's method (reading a mμ) the mean value for the 32 normal 100 mg per 100 ml while the mo method with heating to 90 ° C fo minutes and reading at 630 mμ gave a value of 105 mg per 100 ml. From T it appears that the standard error single determination with the mo method is hardly half as great as Graff's method.

Table II shows that the normal obtained with the anthrone metho lower than a number of normal given in the literature. As it ap probable that the low values may be interference of tryptophan in the protein, the values have been cor from a nomogram according to Tul Keiding (1954). This was construc the following way: A number of sol containing equimolar amounts of gal and mannose were diluted so that corresponded to values of protein-serum hexose between 80—350 m 100 ml with intervals of 10 mg per 1 To each one of these solutions were varying amounts of l-tryptophan. solutions were then treated with anthrone reagent and heated to 90° 14 minutes as described above. Re were taken at 630 mμ and 530 mμ. results were drawn on a nomogram the ratio of 530/630 mμ absorbanc the ordinate and absorbancy at 5 on the abscissa (Fig. 2). By readi absorbancies for serum samples at 53 630 mμ, the values of protein-hexose can be interpolated fro nomogram.

ble I. *Normal values for protein-bound hexose in blood serum analyzed with various modifications of the anthrone method*

fication of the anthrone od.	Number of sera	Mean mg %	Range	Standard deviation	Standard error of the mean mg %	Standard error of a single determination[1] mg %
& co-workers	32	100	82—124	11.3	2.0	5.0
method modified by the author. n time 14 min at 90° C	32	105	87—121	8.0	1.4	2.1
ed anthrone method; ues corrected for trypto- nterference by recovery nents described in text	32	122	102—146	10.0	1.8	2.4
ed anthrone method; the corrected for trypto- nterference from nomo-	32	123	103—146	8.8	1.6	2.5

lculated from the differences between duplicate determinations.

ble II. *Normal values for protein-bound hexose in blood serum given by various investigators.*

Investigator	Reagent	Number of individuals	Mean mg %	Normal range
Lustig & Langer (1931)	Orcinol	73	127	78—156
Seibert et al. (1943)	Carbazole	13	114	88—133
Seibert et al. (1947)	Carbazole	43	103	73—131
Stary et al. (1950)	Orcinol	40	120	93—140
Shetlar (1952)	Tryptophan	43	111	93—127
Berkman et al. (1953)	Anthrone	15	146	119—160

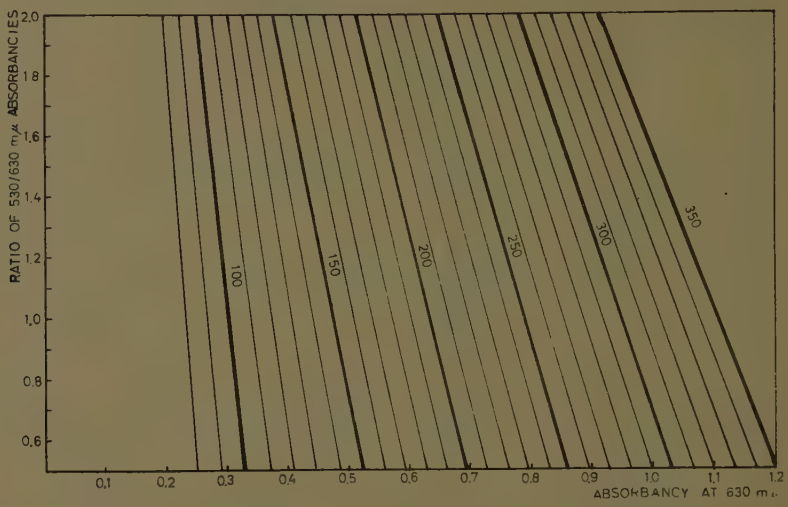
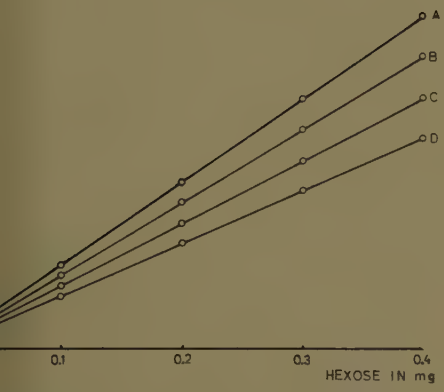


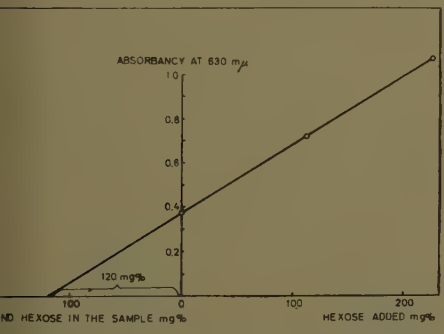
Fig. 2. Nomogram for calulation of protein-bound serum hexose in analyses with the anthrone reagent. The straight lines correspond to values of protein-bound hexose from 80—350 mg % with intervals of 10 mg per 100 ml.

Fig. 3 shows the result of a number of experiments with equimolar galactose-mannose solutions of varying concentrations to which l-tryptophan was added. Plotting the absorbancies at 630 mμ on the ordinate and the amount of hexose on the abscissa the solutions containing a constant amount of tryptophan form straight lines. The curves have less slope than for corresponding samples without tryptophan, and the higher the tryptophan content the less is the slope of the curve. The circumstance that the standard curve for galactose-mannose at constant tryptophan content results in a straight line has been utilized by the author to control the results obtained from the nomogram constructed as described above. Control analyses were made of the 32 normal sera whose content of protein-bound hexose had previously been estimated from the nomogram ethanol precipitates of these, va amounts of an equimolar galactose-ma solution were added, then analyses performed as described above. The r were plotted in diagrams as in Fig. 4 the abscissa are shown, to the right amounts of hexose added, to the lef amounts of protein-bound hexose. O ordinate is shown the absorbancy a mμ. Absorbancy for the preci without addition of hexose is plott 0 mg per 100 ml added hexose. For serum the values plotted in the dia form a straight line and the point this line crosses the x-axis indicate amount of protein-bound hexose i sample. The values found in this agree well, as appears from Table I, the values from the nomogram.



Interference of tryptophan in determination f hexose with the anthrone reagent.

quimolar solution of galactose-mannose
quimolar solution of galactose-mannose
- 0.05 mg tryptophan
quimolar solution of galactose-mannose
- 0.10 mg trypthophan
quimolar solution of galactose-mannose
- 0.20 mg tryptophan



Estimation of protein-bound hexose by experiments (anthrone reagent). Normal No. 11. Protein-bound hexose calculated e above graph 120 mg per 100 ml. Protein-hexose calculated from the nomogram nowed in Fig. 2 121 mg per 100 ml.

average for the 32 normal sera in the first instance was 122 mg per 100 ml in the second, 123 mg per 100 ml.

SUMMARY

Determination of protein-bound hexose in blood serum according to Graff gives values which are too low, due to interference of tryptophan in the serum protein. The extent of this error has been estimated, by recovery experiments and from a nomogram, to reach 15—20 per cent.

By standardizing the reaction conditions with respect to temperature and time the accuracy of the method can be increased from ± 5 to ± 2 per cent.

ACKNOWLEDGMENT

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AINING OF PROTEIN-BOUND SERUM POLYSACCHARIDES IN ELECTROPHORESIS STRIPS

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önwall & Köiw (1952) showed that ıethod worked out by McManus (1946), hkiss (1948) and Gomori (1952) for ing of polysaccharides in fixed tissue arations can be used to stain protein-d serum polysaccharides in paper elec-ıoresis strips. The present investigation undertaken to study further the cability of this method for quantitative ıation of the polysaccharide distribution e various serum protein fractions after r electrophoresis.

METHODS

ıe following technique, which agrees on the with that applied by Grönwall & Köiw, was for electrophoresis, staining and reading the .

r polysaccharide staining 0.016 ml of serum applied to each strip as opposed to 0.006 ml rotein staining. This amount can be increased 025 ml in samples with low polysaccharide nt. Amounts of serum larger than 0.025 ml in poor separation. Whatman I filter paper the most suitable. Electrophoresis was per-ed in a veronal buffer pH 8.6 and ionic gth 0.1 at 0.75 mA. The potential gradient e strip reached about 3.3 volt/cm. Complete ation was attained in about 16 hours. The were dried and fixed at 100° C for 10 es.

Preparation of staining solutions.

Periodic acid: 3.6 g periodic acid A.R. and 1.215 g sodium acetate ($NaAc.3\,H_2O$) are dissolved in 135 ml distilled water and then 300 ml absolute ethanol are added. Solution is usable for 1—2 weeks if kept in a dark place.

Reducing solution: 15 g potassium iodide and 15 g sodium thiosulfate are dissolved in 300 ml distilled water.

Fuchsin sulfite solution: 2 g basic fuchsin (Merck) are dissolved in 400 ml boiling distilled water, cooled to 50° C and filtered. To filtrate add 10 ml 2 N HCl and 4 g potassium metabisulfite. Keep solution in well stoppered bottle in the dark at about 2° C overnight. On following day add 1 g decolorizing carbon, mix and filter immediately. Add about 10 ml 2 N HCl in small amounts until after the last addition the mixture does not turn violet-red when drying spontaneously at room temperature. Keep solution in cool dark place.

Sulfite rinse solution: 4 g potassium metabisulfite are dissolved in 1000 ml distilled water, then add 10 ml concentrated HCl.

Drying, fixing and staining of strips was done as follows:

1. Fixed in absolute ethanol 5—10 minutes.
2. Placed in periodic acid solution 5 minutes.
3. Rinsed carefully in 70 per cent ethanol. Ethanol changed for every strip.
4. To 30 ml of the reducing solution given above was added, in small amounts with stirring, 45 ml absolute ethanol and 0.75 ml 2 N HCl. This solution was prepared immediately before use. Each strip was placed in it for 5 minutes. Fresh solution for every strip.

5. Rinsed carefully twice in 70 per cent ethanol.
6. Placed in fuchsin solution until maximum color developed, about 40 minutes. It was important to control that the strips were properly dampened by the solution.
7. Rinsed 4—5 times in sulfite rinse solution. Rinsing was continued as long as fuchsin sulfite could be washed out of the strip.
8. Dehydrated by dipping in absolute ethanol.

Photometer reading.

Staining of the various sections of the strips has been read directly with a photometer constructed according to Köiw (1953), through a 3 mm wide slit and a green filter, with maximum transmission at 550 mμ at which wave length polysaccharide stain shows maximum absorbtion. To compare with the polysaccharide distribution, an electrophoresis strip for each serum was stained for proteins with "amidoschwartz 10 B" (Bayer).

The polysaccharide stained strips in contrast to the protein stained were not made transparent before taking the readings. For the protein curves the lower absorbancies of the transparent strips are an advantage because all of the values read then fall within the range where the correlation between protein content and absorbancy is indicated by a straight line. However, polysaccahide stained strips with their weaker color give too low and unreliable results when made transparent.

Chemical analyses of the different polysaccharide components were made as follows:

Protein-bound hexose with the anthrone reagent (Graff, Greenspan, Lehman & Holechek, 1951), hexosamine according to a modification of Elson-Morgan's method (Blix, 1948) and sialic acid with Ehrlich's reagent (Werner & Odin, 1952).

RESULTS AND DISCUSSION

Specificity of fuchsin sulfite staining.

Fuchsin sulfite is an aldehyde reagent. In the serum there are no preformed aldehyde groups which give a direct stain with this reagent. The polysaccharides must first be oxidized so that free aldehyde groups can be formed. Suitably the oxidation is carried out with periodic acid. All substances w contain the "1,2-glycol" grouping -CH CHOH-in unsubstituted form (or equivalent structure in which hydr groups are replaced by amino or alkylar groups) or its oxidation product -CH CO- show, after periodic acid oxidation characteristic red-violet stain with fuchsin sulfite reagent. Besides the pro bound polysaccharides there are sev substances in the blood serum which fu these conditions. This is the case glucose, glycogen, and the amino a serine and threonine. These substar however, are washed away from the p during electrophoresis and staining or present in too small amounts to be dete The serine and threonine components serum protein appear to have their hydr amino groups so substituted that they not available to periodic acid oxidation. M of the protein-bound serum polysacchar are washed from the electrophoresis st during staining. This was indicated hexosamine analyses of aliquots of se which were applied as spot tests to f paper strips. The hexosamine content in spots showed no decrease after the st were stained and rinsed as described ab Thus it appears under the conditions of experiment that the reaction with fuc sulfite after oxidation by periodic depends solely on the protein-bound p saccharides in the serum.

The components of the protein-bo serum polysaccharides are the hex galactose and mannose in equim proportions, hexosamine principally glu amine and sialic acid. It should be interest to determine whether all of t

le I. *Limit of identification for dif- t mono- and polysaccharides in spot ests with periodate-fuchsin-sulfite.*

Substance	Limit of identification mg
se	2
gen	0.2
	0.4
an	0.4
ose	1
ose	1
samine	1
	0.2
se	0.1
	No reaction
nine	0.3
	0.3
acid	0.03

analysis were carried out as spot tests according to Hotchkiss:

lution of the sample 0.1 ml.
d 0.025 ml periodic acid solution.
ave 5 minutes.
d 0.05 ml N/10 hydrochloric acid.
d 0.1 ml alcoholic reducing solution. Mix.
d 0.05 ml fuchsin reagent. Leave 15 minutes.

onents contribute equally to the on with fuchsin sulfite. For that reason tests according to Hotchkiss were done rying amounts of a number of mono- polysaccharides. The results are given able I. Thus sialic acid could be ed in quantities of 0.03 mg but ose, mannose and glucosamine first in nts of about 1 mg. From this table it o seen that it is impossible to conclude the limit of identification of the simple onents how the carbohydrates react bound in polysaccharide. Fructose a very strong reaction, while inulin, is built on fructose units, gives a ve reaction with fuchsin sulfite, bly because the linkages between the fructose units are so placed that the polysaccharide lacks adjacent free hydroxyl groups. On the contrary glycogen and starch which are both built on glucose give a stronger reaction than the corresponding amount of free glucose. In the free glucose, partial overoxidation of OH-groups to COOH or CO_2 probably occurs and these groups do not react with fuchsin sulfite.

To determine the parts taken by the different polysaccharide-components in fuchsin sulfite staining, a number of tests with different glycoproteins[1] with varying amounts of hexose, hexosamine and sialic acid were made. The composition of the preparations used is given in Fig. 1. The different glycoproteins were applied to the paper in varying concentrations and were stained with fuchsin sulfite in the same way as paper electrophoresis strips. For all the preparations the limit of identification was about 1 μg computed as the sum of hexose, hexosamine and sialic acid in spite of the widely varying composition of the different components. These experiments suggest that the color intensity in the fuchsin sulfite staining is correlated to the total amount of protein-bound polysaccharide and that the variations in composition of the polysaccharides are of less importance.

To show this quantitatively, hexose, hexosamine and sialic acid analyses were made on some serum protein fractions separated by preparative paper electrophoresis. The loss through incomplete eluation was estimated by comparing the amount of

[1] These substances were kindly placed at my disposal by Prof. Gunnar Blix, Docent Ivar Werner and Dr. Lars Odin of the Department of Medical Chemistry.

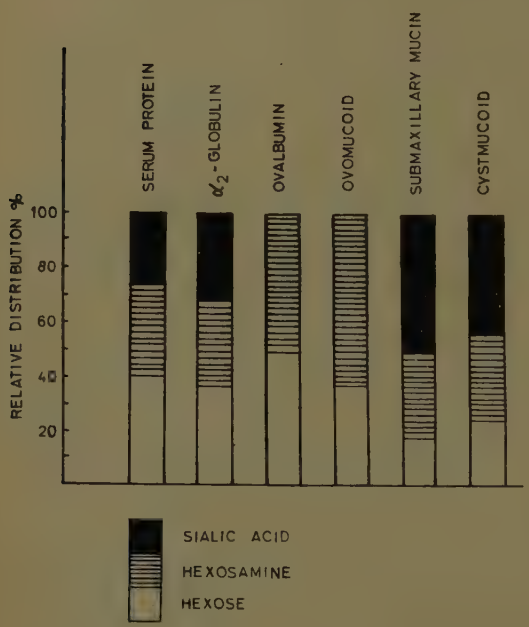

Fig. 1. Distribution of protein-bound hexose, hexosamine, and sialic acid in various glycoproteins, the staining properties of which have been tested with fuchsin sulfite after periodic acid oxidation.
Various amounts of the different substances were applied on filter paper strips which were fixed and stained in the same way as paper electrophoresis strips.
Limit of identification for all glycoproteins in the figure was about 1 μg calculated as the sum of hexose, hexosamine, and sialic acid.

protein in the eluate with the amount of serum protein which was applied to the strips. The values were corrected according to the losses thus found. The relative distribution of protein-bound polysaccharide calculated in this way was compared with the distribution estimated by direct photometric analysis of fuchsin sulfite stained paper electrophoresis strips from the corresponding serum. Satisfactory separation of albumin

Table II. *Comparison between rel distribution of protein-bound serum- saccharide in fuchsin-sulfite-stained ele phoresis strips and relative distributio tained by chemical analyses of protein-b hexose, hexosamine, and sialic acid in trophoretically separated and eluated pr fractions from the same serum.*

Fraction	Relative distribution of polysacch in per cent	
	In fuchsin sulfite-stained electrophoresis strips. Mean value of 4 experiments	In eluated p fractions ca ted as hexo hexosamine sialic aci Mean value experime
Albumin + α_1-globulin	27.3	26.8
α_2-globulin	36.5	36.5
β-globulin	26.8	26.7
γ-globulin	9.4	10.0

and α_1-globulin by preparative paper ele phoresis was not achieved, therefore fractions were analyzed together. Resu these experiments are given in Tabl Even from this it seems possible to cal the relative distribution of polysacchari the various serum protein fraction fuchsin sulfite staining of electroph strips.

Estimation of experimental error in saccharide staining of paper electr phoresis strips.

To determine the error in polysacch staining of electrophoresis strips, 0.0 aliquots of one serum sample were a to 30 strips which were then proces

le III. *Determination of the error of ethod in calculating the relative distri- n of protein-bound polysaccharide in in sulfite-stained electrophoresis strips.*

ction	Number of determinations	Relative distribution of polysaccharide in per cent. Mean	Standard deviation — standard error for a single determination	Standard error in per cent of the mean
umin	30	14.4	2.7	18.8
lobulin	30	14.7	2.6	17.7
lobulin	30	27.9	2.4	8.6
obulin	30	22.3	2.3	10.3
obulin	30	20.6	2.8	13.6

6 ml aliquots of the same normal serum were d to 30 filter paper strips. After electro- is the strips were stained and read with a lectric colorimeter.

ibed above. The results are given in e III. The standard error in per cent e mean for a single determination varied een 8.6—18.8 per cent for the various ons. The largest error was found with ractions which had the least poly- aride content. In pathological cases increased serum polysacchaarides, the seemed to be less because the poly- aride peaks were higher and more ounced.

ive distribution of polysaccharide in different serum protein fractions of normal human material.

e method was also used to determine lative distribution of polysaccharide in fferent serum protein fractions of normal humans, 30 healthy men from 18 to 35 years old. As a comparison, the protein distribution of the same material was also determined by paper electrophoresis. The results are given in Table IV. The standard deviation and standard error of the mean for the polysaccharide fractions are somewhat greater than for the corresponding protein fractions.

Comparison between polysaccharide content in serum and the area of electrophoresis diagram.

For a number of sera with varying normal and pathological polysaccharide content, the sum of the analytical values for protein-bound hexose and hexosamine was compared with the total area enclosed by the polysaccharide curve of the electrophoresis diagram. The correlation was not satisfactory, probably because of the difficulty in obtaining identicial conditions at the various stages each time that staining was done. Therefore the polysaccharide curve can only be used for calculation of the relative distribution of polysaccharide in the serum protein fractions. To obtain the absolute values it is necessary to determine, in addition, the protein-bound hexose and hexosamine content in the serum. In most cases, however, it should be sufficient to estimate only one of these components as the increase in protein-bound hexose in blood serum in pathological conditions seems to parallel a corresponding increase in hexosamine content.

Table IV. *Distribution of protein and protein-bound polysaccharide in normal human serum determined by paper electrophoresis.*

A. *Protein.*

Fraction	Number of determinations	Relative distribution in per cent Mean	Standard deviation	Standard error of the mean
Albumin	30	56.5	3.6	0.66
α_1-globulin	30	4.7	1.0	0.18
α_2-globulin	30	9.6	1.3	0.24
β-globulin	30	14.1	2.0	0.37
γ-globulin	30	15.0	2.1	0.38

The electrophoresis strips were stained with "Amidoschwartz 10 B" and read with a photoelectric colorimeter.

B. Polysaccharide.

Fraction	Number of determinations	Relative distribution in per cent Mean	Standard deviation	Standard error of the mean
Albumin	30	13.1	4.1	0.75
α_1-globulin	30	17.9	3.8	0.70
α_2-globulin	30	27.9	4.7	0.86
β-globulin	30	23.8	3.7	0.68
γ-globulin	30	17.2	4.5	0.82

The electrophoresis strips were stained with periodate-fuchsin-sulfite and read with a photoelectric colorimeter.

SUMMARY

The method of staining protein-bo serum polysaccharide in paper elec phoresis strips developed by Grönwal Köiw (1952) has been investigated respect to its applicability in quantit determination of serum polysacchar This method appears to be useful calculation of the relative distribution of p saccharide in the various serum pro fractions. However, it cannot be used calculating the absolute polysaccha values, but for this purpose it can completed with analyses of protein-bo hexose and hexosamine.

The method has been used to deter the relative distribution of polysaccharid the different serum protein fractions normal humans consisting of 30 healthy from 18 to 35 years old.

ACKNOWLEDGMENT

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PENTAPYRROLIDINIUM AND KIDNEY FUNCTION

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During recent years numerous investigations have been performed on the effect on kidney function of drugs producing ganglionic block. However, as far as I know, the kidney function of individuals who are under the influence of pentapyrrolidinium ('Ansolysen') has not yet been investigated. It would be expected in advance that the effect was almost the same as that of hexamethonium. The purpose of the present study was to examine the effect of pentapyrrolidinium on the glomerular filtration rate, the renal blood flow, and the excretion of salt and water in experiments of either short or longer duration in patients with arterial hypertension.

METHODS

The glomerular filtration rate was determined with inulin clearance, the renal plasma flow with PAH-clearance. Inulin was administered intravenously in a large initial dose and then continuously by the intravenous drip method. Para-aminohippuric acid was administered in a single hypodermic injection, and it was intended to establish a plasma concentration varying from 0.5 to 3 mg per 100 ml. Inulin was determined by the method described by Bojesen (1952), whereas para-aminohippuric acid was measured with Brun's rapid method (1951), modified for measurements in the Beckmann spectrophotometer. The bladder emptied through a multi-eyed catheter, and irr with distilled water and air. In the calculati inulin- and PAH-clearances, corrections were for the delay time from Brun, Hilden & Raas curve (1954). Potassium and sodium in urin plasma were measured in the Beckmann sp photometer, and the potassium values were cor according to Rud's method (1954). Chloride in urine and plasma were determined accord the method of Schales & Schales (1941).

MATERIAL

The study was divided into 4 experi groups:

(1) In 9 hypertensive patients I examine effect of intravenous injection of penta lidium on the inulin- and the PAH-clear output of urine and blood pressure. A control periods of about 10 minutes eac patients received pentapyrrolidinium in nously through the injection tube for the venous drip from 12 to 15 cm from the n

[1] Aided by a grant from P. Carl Petersen's Foundation.

[2] It may be pointed out that intravenous a stration of pentapyrrolidinium is not alto safe. A fall in the blood pressure which wa cult to control was observed in several of ou up to from 20 to 30 minutes after the admi tion had been completed. In one case the became unconscious with 'tobacco panting' 40 minutes after discontinuance of the intra administration; she only recovered after muscular injection of ephedrine. After this we always have l-noradrenaline ready, and we introduced the following measures:

a rule, the experimental periods varied from to 15 minutes; though a few periods lasted to 20 minutes when the output of urine was y low. During the last 1 or 2 hours before during the whole experiment the patients re told to drink much, up to ½ litre per r.

5 patients I examined the inulin- and the H-clearances before the treatment and r from 40 to 75 days of treatment with tapyrrolidinium bitrartrate by mouth.

addition to the investigations mentioned in I observed in 3 of these patients the excre- of sodium, potassium and chloride after avenous injection of pentapyrrolidinium.

e 24-hour urinary excretion of sodium, potas- n and chloride was observed in 7 patients o were given the ordinary low salt diet of department with a known admixture of ium chloride daily and fluid intake *ad tum.*

RESULTS

results are given in Tables I—III and ures 1 and 2. Table I shows that neously with the fall in the blood e intravenous injection of pentapyrro- n causes a considerable reduction of ne flow and some decrease of the inu- d the PAH-clearances. In one case, r, the inulin-clearance rose in spite nsiderably lowered mean blood pres- liastolic blood pressure plus one-third difference between the systolic and c pressures). In 4 cases the filtration increased, in 2 it fell, and in 3 it ed largely unchanged.

ration of pentapyrrolidinium the upper part atient's body is elevated about 30°, and as the fall in the blood pressure commences ction of pentapyrrolidinium is stopped and ent is placed horisontally, so that he is in umbent position both during the control and during those of treatment.

Five patients were examined before and after from 40 to 75 days of treatment with pentapyrrolidinium by mouth. The results are given in Table II, which shows the effect on kidney function and the changes in the eyeground. In 4 patients there was a rise in the glomerular filtration rate of from 10 to 67 per cent, and the renal plasma flow increased by from 9 to 78 per cent. In Case 5 there was considerable anemia at the first examination, presumably owing to a bleeding duodenal ulcer; but at the last examination the hemoglobin percentage was normal. In this patient the glomerular filtration rate and the renal blood flow remained unchanged, whereas the renal plasma flow had fallen considerably at the second examination; this was presumably due to the fact that the amount of plasma in the blood was lower then owing to the greater volume of blood corpuscles.

As already mentioned, pentapyrrolidinium causes a considerable decrease in the output of urine. Table III shows that the excretion of sodium and chloride is reduced at the same time, whereas that of potassium shows only minor changes. Fig. 1 shows the fall in the output of urine and in the sodium excretion in comparison with the control values; it appears that the excretion of sodium showed a greater fall than that of water in these 3 cases.

In the case of administration of pentapyrrolidinium for several days there is during the first few days of treatment a highly varying lowered excretion of sodium chloride and water. Fig. 2 shows the daily output of urine and the excretion of sodium, potassium and chloride in one of the cases before and during treatment with pentapyrrolidinium.

Table I. *Effect of pentapyrrolidinium on excretion of urine, glomerular filtration rate, renal plasma flow, and filtration fraction.*

Patient No.	MBP A	MBP B	UV A	UV B	GFR A	GFR B	RPF A	RPF B	FF A
1. ERU.	159	137	6.7	4.3	80	64	376	229	0.213
2. HPN.	139	95	6.4	0.42	58	38	286	285	0.203
3. DID.	141	90	6.5	0.35	80	33	369	277	0.217
4. JNI.	174	129	4.9	1.0	107	81	404	258	0.265
5. WNI.	160	125	7.5	0.6	118	90	858	672	0.138
6. ELO.	186	130	10.2	0.12	64	32	390	187	0.164
7. OBR.	159	115	4.0	0.3	104	84	638	402	0.163
8. GDY.	158	110	6.4	0.3	80	51	483	354	0.166
9. MNI.	143	109	3.6	0.69	105	113	549	469	0.192

A. Average values in 3 control periods.
B. Average values in from 2 to 4 periods when the blood pressure has been stabilized after intravenous administration of pentapyrrolidinium bitartrate. However, in Case 7 the results are of one period only.

MBP — mean blood pressure.
UV — Urine volume ml/min.
GFR — Glomerular filtration rate in ml/m
RPF — Renal plasma flow in ml/min.
FF — Filtration fraction — GFR/RPF.

Table II. *Renal function examinations before and during treatment with pentapyrrolidinium.*

Patient No.	Date	GFR	RPF	FF	RBF	MBP during clearance determination	Retinal cha
2. HPN.	7/ 7—53	58	286	0.203	469	139	Only arterios
	22/ 9—53	79+36%	467+63%	0.169	742+58%	141	change
3. DID.	5/ 8—53	80	369	0.217	683	141	H E
	15/ 9—53	88+10%	443+20%	0.198	764+12%	123	(E)
10. ENI.	15/ 6—54	42	165	0.255	300	169	H E
	24/ 8—54	70+67%	293+78%	0.239	513+71%	142	(H) (E
11. ICH.	7/ 9—53	53	290	0.183		161	H
	23/10—53	60+13%	317+ 9%	0.190		155	(H)
5. WNI.	4/ 8—53	118	858	0.138	1128	160	H E
	15/ 9—53	114÷ 4%	725÷16%	0.157	1190+ 5%	161	(H)

RBF — Renal blood flow-RPF/1 ÷ hematocrit values.
H — Hemorrhages
E — Exsudates
P — Papilloedema
( ) — Subsiding
Other abbreviations are explained below Table I.

le III. *Changes in the renal excretion of water, sodium, chloride and potassium after intravenous injection of pentapyrrolidinium in 3 cases of arterial hypertension.*

Periods	Urine volume ml/min.	Glomerular filtration rate	Sodium excretion meq./min.	Chloride excretion meq./min.	Potassium excretion meq./min.	Mean blood pressure	
1 2 3	10.2	64	1.06	0.89	0.072	186	Control periods
4	9.0	56	0.79	0.63	0.066	179	4 mg of penta-pyrrolidinium bitartrate intravenously in the course of periods 4, 5 and 6
5	5.9	51	0.38	0.30	0.052	165	
6	1.9	32	0.11	0.10	0.030	150	
7	0.26	29	0.016	0.009	0.023	136	
8	0.11	32	0.008	0.007	0.026	129	
9	0.12	32	0.006	0.000	0.026	131	
1 2 3	4.0	104	0.78	0.70	0.066	159	Control periods
4	6.5	111	0.78	0.72	0.071	170	7 mg of pentapyrrolidinium bitartrate intravenously in the course of periods 4, 5, 6 and 7
5	5.3	93	0.51	0.47	0.060	160	
6	5.7	89	0.28	0.33	0.063	150	
7	6.8	93	0.17	0.19	0.098	143	
8	5.2	69	0.83	0.084	0.049	126	
9	0.45	43	0.015	0.020	0.029	113	
10	0.30	84	0.013	0.024	0.077	115	
1 2 3	3.6	105	0.64	0.54	0.063	143	Control periods
4	9.1	105	0.49	0.47	0.088	131	3 mg of pentapyrrolidinium bitartrate intravenously in the course of periods 4 and 5
5	7.9	91	0.29	0.26	0.078	125	
6	4.0	68	0.12	0.16	0.059	110	
7	0.9	77	0.07	0.08	0.057	103	
8	0.8	106	0.12	0.13	0.094	110	
9	0.4	98	0.05	0.07	0.060	102	
10	0.9	134	0.14	0.14	0.087	111	

DISCUSSION

se investigations show that pentapyrro-
m in intravenous injection exerts
the same effect on kidney function as
ethonium; simultaneously with the
the blood pressure it reduces the renal
flow and the glomerular filtration rate.
ect on the filtration fraction varies like
f hexamethonium (Mills & Moyer, 1953), as it may produce either an increase or a diminution. It is worthy of note that the fall in the blood pressure after intravenous injection sets on much more slowly than that produced by hexamethonium, and persists somewhat longer, as it will hardly have begun to rise again after 1½ or 2 hours.

The rise in the inulin- and the PAH-clearances after treatment for some time with

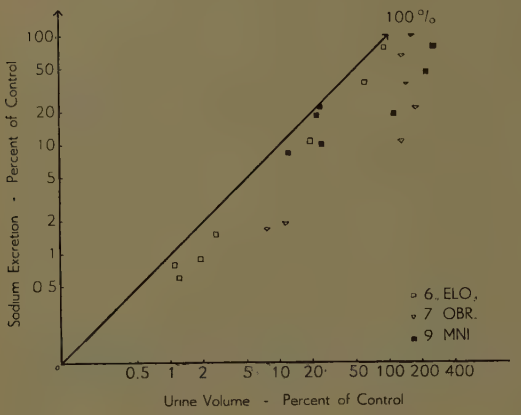

Fig. 1. Output of urine and sodium excretion in 3 hypertensives after pentapyrrolidinium, given in per cent of the control values before the administration.

pentapyrrolidinium may be accidental; Davies & Schock's investigations (1950) showed that such great clearance variations *may* occur spontaneously in examinations of the same person on different days. The rise is, however, most likely to be real, for it was observed in 4 out of 5 cases and the improved kidney function was accompanied by other objective signs of improvement; in 4 cases the retinal changes thus subsided. At the second examination all patients stated that they felt better. The cause of the improved kidney function is unknown, but no doubt the improvement suggests that some of the changes in the renal vessels are reversible and, like the retinal changes, subside when the blood pressure is lowered.

McQueen (1952) and Moyer & Mills (1953) have previously shown that a single dose of hexamethonium reduces the excretion of both sodium and water. This reduction is much more pronounced than that of the renal blood flow and of the glomerular

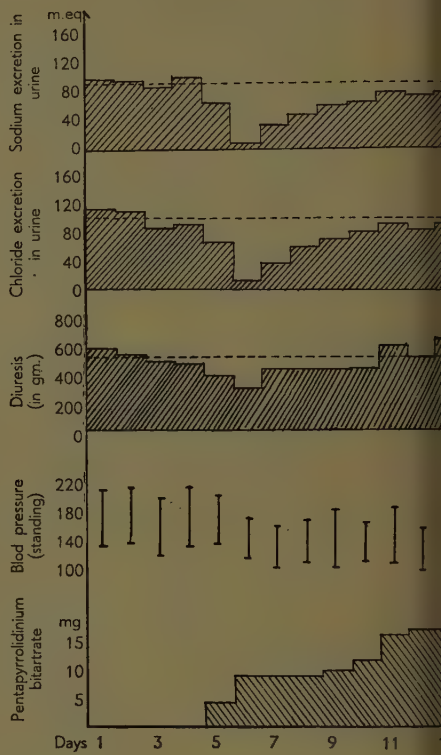

Fig. 2. 24-hour output of urine and urinary tion of sodium chloride in a hypertensive (6 before and during treatment with pentapyrroli hypodermically t.i.d. During the whole expe the patient received a low salt diet plus 5 salt daily, in all about 6 gm of salt dai

filtration rate; the investigations des here (Tables I and III) show that pyrrolidinium exerts the same effect. & Mills found that during treatment hexamethonium the retention of sal water is of almost the same order of m tude in large diuresis, but when the of urine falls below 20 to 30 per cent control values the retention of water i pronounced. In my 3 cases the retent salt was greatest, regardless of the size

is. In Cases 7 and 9 this may be due fact that the intake of water began hat late, so that the diuresis was in- ng when the patients received the in- n of pentapyrrolidinium; this, however, ot apply to Case 6. In all 3 cases the ion of salt decreased simultaneously he ultrafiltration.

already mentioned, the 24-hour excre- f sodium chloride and water decreases treatment of hypertension with penta- lidinium is instituted. This retention e so pronounced in a few cases that atients develop symptoms of heart e: Oedemas, ascites, pulmonary conge- and, occasionally, cardiac asthma (Røn- essen 1955). This salt and water reten- hardly due to a specific effect of penta- lidinium, as it may also occur after administration of reserpine (Crone, p, Hilden & Kroghsgaard), and pro- atrin is known to cause retention of (Meilman, 1953). At first glance it probable that the salt and water reten- ollowing a single dose of pentapyrrolidi- is exclusively of renal origin, and ps determined by lowered ultrafiltra- with preserved or even increased ar back resorption. The latter is pos- supported by the fact that a lowered tion of salt may sometimes be seen, n cases where the glomerular filtration has remained unchanged (Moyer &). The salt retention in long-term treat- may possibly be of the same origin. It rs from Table III that during the last xperimental periods Cases 6 and 7 ex- l per minute such small quantities of salt he excretion corresponded to only from 20 meq. in 24 hours, *i. e.* only a fraction of the quantity administered daily. On the other hand, the effect on renal function and on the salt and water metabolism of the organism resembles in many respects the influence seen in heart failure: reduction of the renal plasma flow and of the glomerular filtration rate, in many cases with a rise in the filtration fraction; furthermore, retention of sodium chloride and water, and in many cases treated for a prolonged period a high nocturnal diuresis and a very low output of urine by day. It is quite possible that these patients, like those with heart failure, produce too much salt-retaining hormone. This possibility might be confirmed by examining whether extracts of urine from which pentapyrrolidinium has been removed cause salt retention in animals.

SUMMARY

In addition to the fall in blood pressure, pentapyrrolidinium, like hexamethonium, causes a decrease of the renal blood flow and of the ultrafiltration and reduces the output of urine and the excretion of sodium chloride. In spite of this immediate effect, it may be seen after prolonged treatment in some cases that the inulin- and the PAH-clearances rise; this is possibly indicative of a real improvement of the kidney function. In a few cases, treatment with pentapyrrolidinium may lead to heart failure due to the water- and salt-retaining effect of the drug.

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THE HEMODYNAMIC EFFECT OF RESTORING NORMAL SINUS RHYTHM IN PATIENTS WITH AURICULAR FIBRILLATION

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ecent years cardiologists have shown reasing interest in the conversion of ar fibrillation to normal sinus rhythm use of quinidine. The main goal in ng sinus rhythm has been the preven- embolish, with its disastrous results. ttention has been paid to the effect the restoration of sinus rhythm has circulation.

problem may be attacked in 3 ways. nay, in the first place, compare the tory dynamics of patients with ar fibrillation with those of patients inus rhythm. Ferrer, Harvey, Cath- Tournand & Richards (1952) found, atients with mitral stenosis and auri- fibrillation, a lower cardiac output n 7 patients who also had mitral s, but showed sinus rhythm. Wade, , Eliasch, Gidlund & Lagerlöf (1952) red 2 groups of patients with mitral r disease: one group of 6 patients auricular fibrillation and one of 9 s with sinus rhythm. The patients auricular fibrillation had a lower output and stroke volume and lower re in the pulmonary circulation, but er arterio-venous oxygen difference ose sinus rhythm.

disadvantage of this method of rison is that it is difficult to find two of patients who are similar in all respects other than that of heart rhythm. Auricular fibrillation occurs most commonly in patients with advanced congestive failure and is usually an indication of increasing dilatation of the atria.

It is therefore more satisfactory to study the same group before and after conversion of auricular fibrillation to sinus rhythm.

Kory & Meneely (1951) measured the cardiac output at rest and during exercise in 6 patients with auricular fibrillation. These patients had a low cardiac output at rest (cardiac index 1.89 liters/min/m^2 body surface). After conversion to sinus rhythm the mean cardiac output showed a rise of 43 per cent both at rest and during exercise.

Hecht, Osher & Samuels (1951) similarly investigated 15 patients before and after conversion to sinus rhythm. Their patients reacted differently with respect to circulatory dynamics: one group showed no difference in cardiac output before and after conversion, a second group showed a distinct increase in the cardiac output both at rest and during exercise, while the third group showed an increase in cardiac output only during exercise. The mean cardiac output in these patients showed the greatest increase during exercise. At the same time there was a decrease in the high arterio-venous oxygen difference. The pressures in

the pulmonary circulation did not undergo any notable change.

Reeve-Hansen, McClendon & Kinsman (1952) have made a more thorough investigation of the circulation in 14 patients with auricular fibrillation both before and after conversion to sinus rhythm. Of these, 11 showed an increase in cardiac output while 3 showed a slight fall. During exercise the average increase in the cardiac output in 9 patients with auricular fibrillation was 17.9 per cent. After conversion to sinus rhythm the average increase in the cardiac output in 7 patients was 22.9 per cent. After conversion to sinus rhythm the average pulmonary blood volume fell from 1627 to 1453 ml. Nine patients showed a fall in the total peripheral resistance, while 4 showed a slight increase. Eight patients showed a fall in mean brachial artery blood pressure, while 5 showed a rise. Of 6 patients, 4 showed a fall in mean right atrial pressure, one no change and one an increase. Similarly there was a fall in mean pulmonary artery pressure in 4 patients and an increase in 2 patients.

The effect of quinidine *per se* on the circulation has been studied by Ferrer, Harvey, Werkö, Dresdale, Cournand & Richards (1948), who studied the hemodynamics after a single dose of 0.8 gm of quinidine in 8 normal subjects and in 12 subjects with heart disease. They observed a fall in systemic blood pressure and peripheral resistance in half of the normal subjects and in 8 of the heart patients. In the latter group 5 showed a fall the pressure in the right ventricle, 2 a fall in the cardiac output, while 5 showed an increase. Harvey, Ferrer, Cathcart, Richards & Cournand (1949) observed, in one patient with heart failure, a f the pulmonary artery pressure and blood pressure and peripheral resistanc an increase in cardiac output and volume following a single dose of 0. quinidine.

MATERIAL AND METHOD

During a clinical study of quinidine tre of established auricular fibrillation in 74 p (Storstein & Tveten 1955), we made some ob tions of the hemodynamics of 5 patients befo after conversion to sinus rhythm. The p were examined just prior to the commencem quinidine treatment by the technique of r catheterisation, as previously described (St 1952). Shortly after conversion to sinus r the patients were reexamined by the technique. A sixth patient, who was treated same way, but did not convert, served as a c

RESULTS

The results are shown in Table I.

We see in three patients a distinct f the high arterio-venous oxygen diffe and in two no change. Similarly patients showed an increase in the ca output, while two showed no change. stroke volume was unchanged in patients, showed a fall in two and a r only one patient. The pressure in the monary circulation showed a fall in on no change in the other 2 patients in who pressure was measured. On an av there was a fall in the arterio-venous ox difference, in the stroke volume and i pressure in the pulmonary circulation, v there was a rise in the cardiac output. patient who served as a control show change in these values apart fro increase in the stroke volume.

b l e I. *Hemodynamics before and after conversion of auricular fibrillation to sinus rhythm.*

Sex Age	A—V O_2diff. ml/min. Before	After	Cardiac output lit/min. Before	After	Stroke volume ml Before	After	Pulm. art. press. mm Hg Before	After	PCV press. mm Hg Before	After
F. 50	67.4	47.1	3.1	3.9	39	34	60	30	29	20
M. 33	81.9	68.5	2.7	3.3	54	41	40	48	32	34
F. 60	62.7	48.7	4.0	4.7	40	47	16	-	15	-
M. 71	54.2	55.9	3.7	3.7	92	62	11	11	7	7
F. 62	31.6	32.7	7.6	7.3	95	91	-	-	-	-
	59.6	50.6	4.2	4.5	64	56	37	30	23	20
ol: M. 59	68.3	68.4	3.1	2.9	39	58	26	22	19	19

DISCUSSION

e results of this study are consistent those of Kory & Meneely, Hecht *et al.* Reeve-Hansen *et al.*, showing as they n improvement on the whole in the lation, with a fall in the high arterio- us oxygen difference and a corres- ing increase in the cardiac output and l in the pressure in the pulmonary lation.

ere could be various explanations of results: they may be due to the resump- of the atrial systole, to the direct effect inidine on the heart muscle or to the heral action of quinidine. The contri- n of atrial systole to the stroke volume fficult to evaluate, but it is conceivable regular heart action with equal diastolic time will, among other things, improve circulation in the heart itself and then the contractile force of the heart e. The results obtained by Ferrer *et al.* by Harvey *et al.* by a single dose of quinidine are reminiscent of those changes in hemodynamics following quinidine treatment, observed in this study and in others mentioned above, and may indicate that the chief effect of quinidine on the circulation is due to its peripheral action of dilatation of the blood vessels and reduction of the total peripheral resistance.

SUMMARY

Cardiac catheterisation studies in 5 patients with auricular fibrillation, both prior to and following restoration of normal sinus rhythm, showed a mean fall in the arterio-venous oxygen difference and a corresponding rise in the cardiac output, a slight fall in stroke volume and a fall of the pressure in the pulmonary circulation.

This effect could be due to the restoration of sinus rhythm, but is more probably due to the peripheral action of quinidine on the blood vessels.

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MICROMETHOD FOR OWREN'S PROTHROMBIN DETERMINATION

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some years we have used the method
ren (Owren & Aas, 1951) for pro-
in determination as a daily routine
l in dicumarol therapy and have
it very reliable and accurate. In not
patients, however, the necessity for
venous punctures has proved a great
enience, which has even in some cases
the continuance of the dicumarol
y impossible. We therefore have
o make the determination in capillary
and after some experimenting we
ome to a technique, that in our hands
ven very satisfactory results.[1]

r rubbing with ether a fresh cut is
in the ear lobule with a sharp knife,
t it bleeds well without pressing. A
wad of cotton is placed on it for a
and then removed. The first three
hereafter are wiped away and the
of the pipette is begun. In a 0.1 cc
with a mark at 0.01 cc, 2 per cent
um oxalate solution is drawn up to
c and then blood to 0.1 cc. It is
nended to use a pipette with a curved
e last ½ cm being curved to about
nd not too narrow a diameter (the distance from the point to 0.1 cc in our pipettes is about 13 cm), as this facilitates the mixing of blood and oxalate solution in the pipette. The mixture is at once blown out into a test tube of suitable size containing 0.52 cc of Owren's buffer solution (Owren & Aas, 1951) and mixed thoroughly with this by swinging the tube for half a minute. The tube is then placed in ice-water (perhaps not strictly necessary) till the determination takes place. From this mixture of blood and Owren's buffer, 0.2 cc are pipetted off for Owren's prothrombin determination (P & P method) in the usual way after taking care that the blood corpuscles are evenly suspended in the fluid. If the patient's hemoglobin percentage is normal (for eventual corrections see below), these 0.2 cc contain exactly the same amount of plasma as the 0.2 cc of plasma dilution used in the ordinary Owren method. The blood corpuscles do not influence the result and the coloring they give is rather an advantage.

In our hospital we have a central laboratory which makes most of the prothrombin determinations (Owren's method). Besides each department has its own small laboratory for simpler routine work. In our department (Medical Department B) we have for years done our own prothrombin determi-

er the termination of our experiments a
at different micromethod has been publi-
Lund & Dyggve (1954) and Dyggve &
954).

Table I. *Prothrombin in per cent of normal (patients with normal hemoglobin values).*

Pat.	Date	Ear-blood	Plasma B	Plasma C	Pat.	Date	Ear-blood	Plasma B
A	20/8	24	27	25	D	19/8	37	37
	21/8	32	30	32		20/8	36	43
	23/8	24	23	22		21/8	27	28
	24/8	19	20	18		23/8	23	23
	25/8	18	17	15		24/8	27	27
	26/8	15	19	16		26/8	37	39
	27/8	13	15	15		27/8	66	66
	28/8	14	15	13		28/8	73	70
B	2/9	18½	17	16		30/8	77	78
	3/9	11	11	9		31/8	75	88
	4/9	8	6	6	E	23/8	23	22
	6/9	21	19½	16		24/8	28	25
C	21/8	18	18	18		30/8	15½	14½
	23/8	16	14	12		31/8	12	11
	24/8	23	17	17		1/9	6½	6½
	25/8	29	24	26		2/9	8	7
	30/8	23	20	18		3/9	7	6
	31/8	21	19	18		4/9	6	5
	2/9	12	10½	10		6/9	24	22
	3/9	7½	5	6	F	19/8	62	67
	4/9	5½	4½	5		20/8	76	75
	6/9	16	15	15				

nations, and all of the determinations mentioned in this paper (with the exception of the plasma controls made in the central laboratory and clearly to be seen as such in the tables) have been made by one of us (E.S.) personally, who has long experience with different methods of prothrombin determination. As soon as our initial work was finished and we had become convinced of the reliability of the technique, we established a close collaboration with the central laboratory in order to have a reliable and independent control.[2]

The blood samples from the ear lobule have been taken in the morning soon after the central laboratory had taken its ve puncture. We have always taken 2 sa from the same cut, each in its seaprate and examined both of them. These d determinations have always been in close accord. The values given in the t are the mean values of these double d minations. The determinations have carried out as soon as possible afte taking of the blood, that is to say bet ¼ and 1 hour after (excepting Table see below), blood samples from up to 5 patients being taken by the same p (E.S.) before starting the determina The taking of the blood requires about 5 minutes for each patient. For 5 pa it is finished in less than half an hour.

[2] We are very much indebted to the chief of the Central Laboratory, Dr. G. Brun, for kind assistance.

le II. *Prothrombin in per cent of normal (hemoglobin not normal, correction).*

Hemo-glob. per cent	Date	Ear-blood		Plasma	
		Found	Correct	B	C
79	19/8	31	28	28	26
	20/8	20	18	22	19
	21/8	20	18	20	16
	23/8	16	15	16	15
	24/8	17	16	12	12
	26/8	19	17	18	17
	30/8	70	63	57	68
	31/8	61	55	58	62
	7/9	71	64	58	68
78	25/8	70	63	68	75
75	7/9	74	65	52	68
72	7/9	78	67	66	80
106	28/8	15	15	16	14
	30/5	20	20	18	23
	7/9	65	67	68	73
111	21/8	53	56	55	62

merous determinations have also been on the same samples after standing arious lengths of time (½—5 hours) -water in the laboratory. This standing o appreciable influence upon the results es III and IV).

comparison, prothrombin deter- ions (double) in plasma (standard n technique (Owren & Aas 1951)) always been made both by ourselves y the central laboratory. The central tory made its venous puncture in the ng, and the plasma then was shared en our laboratory and the central tory. In our laboratory the plasma ot examined until 3 or 4 hours later in ice-water), in the central laboratory lly somewhat earlier. All reagents been given to us daily from the central tory from the portions that were used there that same day, so that any difference in this respect is excluded. Tables I and II show our values (in per cent of normal) for prothrombin in ear blood and our values for prothrombin in venous plasma (B), compared with those of the central laboratory (C).

In comparing capillary blood with plasma a correction should be made for the hemoglobin percentage, as in anemia the 0.1 cc blood contains a greater plasma volume and the prothrombin values consequently will turn out too high. We have made a series of hematocrit determinations on venous blood mixed up with oxalate solution in the proportion 9 + 1 in patients with various hemoglobin percentages, and thereby we find at 100 — 80 — 60 per cent hemoglobin (Sicca method) a plasma volume (plasma + oxalate solution) of 62 — 70 — 78 per cent. This means that for each 10 per cent that

Table III. *Prothrombin in per cent of normal after standing of the samples for some time.*

5—10 min.	½ h.	1 h.	1½ h.	2 h.	2½ h.	3 h
26		29	35			
18	24	25				
41	44	56	52			
51	52	51		48		
47	41	37		38		
25	25	30	29	29		
24	24	24			24	24
26	27	29	28			
26	31	31		32		
27	28					
29	30	30	30	32		
19	21	22				
28	28	30				
21	22			26		
23	25			25		
33	33	33				
48	48	51	54	50		

Table IV. *Prothrombin in per cent of normal after standing of the samples for some time.*

¼—1 h.	1½—2 h.	3—3½ h.	4½—5 h.	¼—1 h.	3—3½ h.	4½—5 h.
31	33		34	24	30	33
62	62		70	36	40	38
37	39		38	23		25
20		25	27	24		25
76		82	84	23	24	
53	57			16		17
12	13			16		17
6½	8			23	26	
12	11			17	19½	
8	9			28	32	
7	8			19	21	
11	13			27	31	
7½	7			18	20	
5½	6			29	31	
6	5½			70	72	
78	83			15	17	

Table V. *Comparison of prothrombin determination in samples with and without clot formation (prothrombin values in per cent of normal).*

Patient	Date	Time before determination	Without clot	With clot
9245	31/8	¾ hour	75	68
»	»	1½ hour	78	75
9226	20/8	½ hour	20	23
»	»	3 hours	25	28
8535	20/8	3 hours	82	90
10298	31/8	1½ hour	23	21

the hemoglobin value is below 100 we should subtract about 5 per cent from the prothrombin value found (for instance hemoglobin 70 per cent, prothrombin value found 40 per cent, corrected 34 per cent). In Table II both the values directly found and the corrected values are given.

How the standing of the samples for some time in the laboratory (in ice-water) influences the results is seen in Table and IV. In Table III we have only blood from one single patient, and this has been brought to the laboratory wi any delay and examined at once, so tha first prothrombin determination coul made about 5 to 10 minutes after the t of the blood. The values in Table IV repetitions of determinations given in I and II, so that the values in the column (¼—1 hour) are those from T I and II (uncorrected). When in instances several repetitions have been formed, this has been made possibl taking blood samples of double size (0 pipette instead of 0.1 cc).

It has happened a few times that a clot has formed in one of the two sam taken from a patient. Then we have rem the clot, made our prothrombin deter tion and compared it with the other sa (Table V). In two cases, 9245 and we could even repeat both determina after some time's standing of the sampl the laboratory. It will be seen that the formation — contrary to our expectatio has not appreciably influenced the re

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BLOOD VOLUME STUDIES ON CANDIDATES FOR THORACIC SURGERY

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the last few years numerous authors stressed the insufficiency of routine ninations of hemoglobin, erythrocyte , hematocrit and plasma proteins in praisement of the general and circula- state of candidates for operation. At ame time the value of blood volume nination in addition to the above men- l estimations has been firmly establi- (Beling, Bosch & Morton, 1950; leton & Finley, 1952; Reich & Wag- 1950). Information of the circulating volume is specially valuable in thoracic ry because of the common heavy opera- lood loss (some data from the literature llected in Table I).

e purpose of this investigation is to ate the relationship in patients who are submitted to thoracic surgery between nination of hemoglobin percentage, rocyte count and plasma proteins on and and cell and plasma volumes on her. The patients were selected among referred to the department of thoracic ry for operative treatment. Only ts with normal hemoglobin, erythro- count and plasma proteins were ed. There were, however, a few ions from this rule; these will be ac- ed for and discussed later.

METHODS

Hemoglobin was estimated by the Autenrieth-Königsberger method. One hundred per cent Hb was equivalent to 16 g. In men 85 per cent (13.6 g) and in women 80 per cent (12.8 g) were chosen as the lower limit of the normal range. *Erythrocyte count* was considered normal above 4.5 mill/mm^3 in men and 4.0 mill/mm^3 in women. Plasma proteins were calculated as the total amount of proteins, the lower limit chosen as 7.0 per cent.

Plasma volume (PV) was determined with the Evans' blue dye (T-1824) method, devised by Gibson & Evans (1937) and later modified by Gregersen (1944) and von Porat (1951). In the present investigation the later modification was applied. The Evan's blue solution was prepared in the hospital dispensary using dye from the Eastman Kodak Company. For details the reader is referred to the original paper.

In the literature the accuracy of the Evan's blue method has been critically discussed (see Whiting & Hotz, 1953; Freinkel, Schreiner & Athens, 1953; Schultz, Hammarsten, Heller & Ebert, 1953; Mayerson, Lyons, Parsons, Nieset & Trautman, 1948). Most authors, however, agree that the accuracy of the method is sufficient for practical clinical purposes; it is said to ascertain changes of over 5 per cent of the PV (Gibson & Evans, 1937). Since, additionally, it is a simple, fast and cheap method I can see no reason to abandon it in favour of more arduous and expensive methods.

Cell Volume (CV) was calculated according to the formula

$$CV = \frac{PV \times Hct}{100 - Hct}$$

Table I.

Operation	Authors	Blood Loss (cc) Range	Average
Thoracoplasty, 1st stage	White & Buxton (1942)		700
	Miller, Gibbon & Allbritten (1949)	406-1885	734
	Stewart & Rourke (1938)	400-900	
	Forsee, Schmidt, Holmes, Jenkins, Huey & Salyer (1952)		973
	Allbritten, Lipshutz, Miller & Gibbon (1950)	467-1407	855
	Cole & Lougheed (1954)	335-1708	932
Thoracoplasty, 2nd stage	Forsee *et al.* (1952)		755
	Allbritten *et al.* (1950)	110-990	404
	Cole & Lougheed (1954)	329-1360	788
Thoracotomy	Allbritten *et al.* (1950)	364-1679	1074
Esophagectomy	Allbritten *et al.* (1950)	1284-2570	1876
Pneumonectomy	Wangensteen *et al.*		1324
	Allbritten *et al.* (1950)	684-3597	1915
	Cole & Lougheed (1954)	400-2930	1051
Lobectomy	Wangensteen *et al.*		1324
	Allbritten *et al.* (1950)	700-2919	1778
	Cole & Lougheed (1954)	329-3066	1135
	White & Buxton (1942)		1500

Hematocrit (Hct) was determined with a glass capillary tube spun in a centrifuge for 30 min. at 3000 r.p.m. (diameter 150 mm). Using this technique a certain amount of plasma still adheres to the cell surfaces. The observed Hct for this reason was multiplied with a factor 0.96 (Root, Roughton & Gregersen, 1946).

Total Blood Volume (TV) was calculated as the sum of PV and CV.

As normal values for TV, PV and CV r tively we adopted TV = 85 × body weight PV = 45 × body weight in kg and CV = body weight in kg (Beling, Bosch & M 1950). No corrections for age, sex or obesi suggested by Randall (1953) were made. above formulae thus were used for the calcu of the "normal" or "optimal" values in the t Values within the range of ± 10 per cent o calculated optimal value were considered as no Control experiments on healthy medical stude both sexes gave results in close accordance these values.

RESULTS

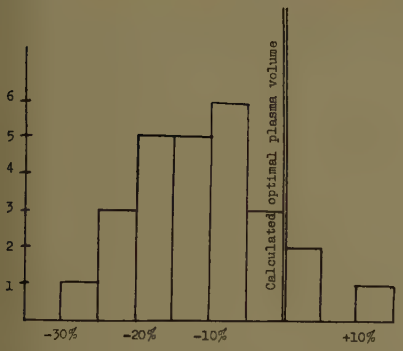
A. *Tuberculosis.*

The results of PV and CV estimatio 26 tuberculous patients are shown in grams 1 and 2. In 21 out of 26 patients per cent) suffering from advanced pulmo tuberculosis the TV was found t markedly decreased, *i. e.* less than 90 cent of the optimal value. Again, in these cases the decrease was greater 20 per cent. As is evident from the grams, both PV and CV are mark decreased with only a few values withi normal range. In diagram 2 it is seen only one patient had a CV on the po side of the "optimal" value line.

B. *Non-tuberculous cases.*

Results obtained from patients suff from diseases of the heart and large ve are recorded in Table II. As coul expected there is a rather wide range a the observed values.

In 8 cases of mitral stenosis there wide variance in the PV and CV value far as it is possible to judge from small series, the degree of PV or CV d cannot be correlated to any other sig



Distribution of plasma volumes in 26 cases of pulmonary tuberculosis.

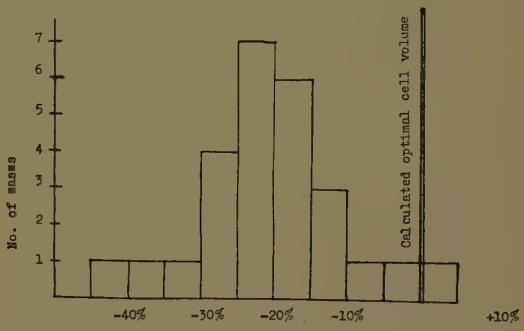


Fig: 2. Distribution of cell volumes in 26 cases of pulmonary tuberculosis.

om of the patient's circulatory state. cement of blood and other fluids and after commissurotomy for mitral is often presents difficulties. Judging the wide range of values it is obvious uch replacement must not be given ing to standard procedures. Repeated volume estimations proved to be of most value in this respect.

cases of essential hypertension TV lly was within fairly reasonable As is seen in Table II, however, may be a marked decrease of CV.

advanced cases of constrictive rditis, plasma proteins most often are rmal because of impaired liver func- The 3 cases presented here are also ions from the rule to use only patients ormal plasma proteins in the present gation. Hemoglobin and erythrocyte however, were normal. In all 3 cases PV and CV were roughly within limits. Also in patent ductus sus cases blood volume was normal. ther exception from the rule stated under "Methods" to use only patients with normal blood values is provided in some cases of the tetralogy of Fallot. Three of the four patients had symptomatic polycytemia, the hemoglobin in some cases exceeding 19 grams. This is reflected in the pathologically increased CV whereas PV is normal. Finally, in two cases of coarction of the aorta, both CV and PV were somewhat decreased.

In Table III some diseases of the esophagus are recorded. As could be expected the difference between calculated and observed values is less pronounced in benign achalasia of the esophagus than in carcinoma. It is, however, clearly demonstrated that even in the former disease the PV and CV deficit may be of high pathological significance.

Most cases of pulmonary cancer suffer from anemia on admission to the department; hence the number of cases presented here is restricted to six. It is notable that PV as a rule is roughly normal whereas CV is decreased.

Table II.

Diagnosis	No. of cases	Cell volume in per cent of normal					Plasma volume in per of normal			
		<80	80—90	90—110	110—120	>120	<80	80—90	90—110	110—
Mitral stenosis	8	2	3	3	0	0	2	1	5	0
Essential hypertension ...	7	2	3	2	0	0	0	1	5	1
Constrictive pericarditis	3	0	0	2	1	0	0	0	3	0
Patent ductus arteriosus	3	0	0	2	1	0	0	0	3	0
Tetralogy of Fallot	4	0	0	0	4	0	0	0	4	0
Coarctation of aorta	2	0	2	0	0	0	0	2	0	0
All cases	27	4	8	9	6	0	2	4	20	1

Table III.

Diagnosis	No. of cases	Cell volume in per cent of normal					Plasma volume in per of normal			
		<80	80—90	90—110	110—120	>120	<80	80—90	90—110	110—
Carcinoma of the esophagus	6	3	3	0	0	0	1	3	2	0
Achalasia of the esophagus	7	1	3	3	0	0	1	1	5	0
Carcinoma of the lung	6	0	4	2	0	0	0	1	5	0

COMMENTS

Earlier reports on blood volume in pulmonary tuberculosis are sparse. Allbritten, Lipschutz & Miller (1950) investigated 10 patients and found that all except one had a decreased blood volume, the average decrease being 15.6 per cent. Berlin, Rowles, Hyde, Parsons, Lawrence & Port (1951) found a reduction of approximately 12.4 per cent of the CV in males and 18.5 per cent in females. Cornet & Duhamel (1952) reported on PV and CV in three groups of patients. They found a connection between the degree of decrease of blood volume and the severity of the illness. In this country von Porat (1951) investigated the PV of tuberculous patients of different ages. In both sexes there was a statistically significant reduction of PV and CV. He also stressed that tuberculous patients may have false normal hemoglobin and red cell values Porat summarizes: "these results are portant above all in connection with tub lous cases in which thoracoplasty opera are to be carried out".

The present investigation confirms results presented by these authors. what is the reason for the decrea circulating blood volume? Several au (Clark, Nelson, Lyons, Mayerson Decamp, 1947; Varco, 1946; Greg 1944; Beling, Bosch & Morton, showed that blood volume is decreas chronic and debilitating diseases other tuberculosis. In some cases dehyd may be responsible for the decrease in this is, however, not the rule in tubercu patients. All patients in these series e one were referred directly from san where they had been subjected to

ng therapy including high caloric food and extensive bed rest. Under these ions with very restricted muscular se the demands on the blood cell cing organs diminish and less erythro- are poured out. Thus often the sana- n patient has a qualitatively excellent whereas it is present in inadequate nts.

e conclusion would be that earlier tion of candidates for tuberculosis y is necessary. A period of cautious lar training under medical supervision e admission to the surgical service no should benefit the patient's CV V.

e wide variance in the results recorded patients with cardiovascular diseases lines earlier statements concerning the lness of blood volume estimation in ndividual case before operation. It is ossible to predict the degree of PV V changes by Hct, red cell count and a protein estimation only (Templeton nley, 1952; Reich & Wagner, 1950; n, Mayerson, Clark & Lyons, 1948; ns, Williams & Forsythe, 1952).

vis & Mayerson in 1948 reported on a ng coincidence between the pre-opera- CV and the ultimate prognosis of athectomized patients with essential tension. They felt that patients with n CV had better prognosis as to their tension after sympathectomy than had nts with low CV. The too small number es and the short observation time does ermit any conclusions to be made in espect. It is, however, to be hoped that er studies on these matters may be up later.

Kyllönen (1954) recently reported on blood volume studies in 44 cases of bronchial carcinoma. He found an average increase of PV of 7.3 per cent. As stated in Table III, the PV in this material also is roughly normal. On the other hand Templeton & Finley found an average deficit of 1000 cc from the calculated TV in 12 cases of cancer of the lung, the Hct in all but one being normal. Also in cases of carcinoma it is obvious that CV estimation is of particular value: firstly a disguised anemia may be discovered, and secondly, the imminent danger of overloading the remaining lung after operation may be prevented.

SUMMARY

1. Studies on red cell volume and plasma volume have been undertaken on patients with normal hemoglobin, red cell count and plasma protein values, admitted for thoracic surgery.

2. In cases of pulmonary tuberculosis both plasma and cell volume as a rule are significantly decreased. The implications of these findings are discussed.

3. There is a wide variance in the blood volume state in cases of disease of the heart and/or the large vessels.

4. Cell and plasma volume are usually lowered in carcinoma of the esophagus and lung and may be decreased in achalasia of the esophagus.

5. Earlier statements concerning the necessity of plasma and cell volume estimations as a routine method before surgery are emphasized.

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LECTROCHEMICAL MEASUREMENT OF DISSOLVED OXYGEN IN BIOLOGICAL FLUIDS

DESCRIPTION OF A SIMPLE MICROAPPARATUS

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simple and exact analytic method for etermination of dissolved oxygen in ical fluids, *e. g.* blood, saliva, milk and , is still lacking. The volumetric ods of Krogh (1908), van Slyke or ander (1947) are rather troublesome similar to Winkler's chemical method), require constant testing and quent time-consuming analyses. Direct, and continual oxygen determinations ody fluids have only been obtained by larographic method as used by Bartels), and by the electrochemical methods avies & Brinch (1942) and Tödt & (1952). While the dropping mercury ode requires a complicated apparatus, only able to record changes in oxygen n of about 400 μg O_2/1 solution, irements a thousand times more ive have been registered with the elec- mical method. The electrochemical od has recently been used for continual irement of oxygen consumption in synthesis (Todt, Damasche & Rotbühr, . The electrode vessels contained from 350 ml solution (Chlorella in phos- buffer) which was stirred by means pecial device.

far as is known, micro-electrode ves- or measurements in resting electrolyte ons below quantities of 5 ml have not yet been mentioned in the literature. In the following, a simple apparatus for the measurement of oxygen dissolved in biological fluids in quantities of 3 to 4 ml will be described.

PRINCIPLE AND CONSTRUCTION OF THE APPARATUS

The principle of electrochemical determination of oxygen has been described by Tödt in several papers (1942, 1947, 1953). The apparatus comprises a galvanic cell with a polarisable and an unpolarisable electrode immersed in an electrolyte, giving currents proportional to the oxygen tension of the solution. The process is as follows: The metal ions are dissolved on the attackable electrode, which acts as an anode. The liberated electrons form a circuit in the galvanic cell, and reduce the molecular oxygen at the cathode, the current being a quantitative expression of the cathode oxygen reduction, and consequently of the oxygen content of the electrolyte. A necessary condition is, however, that suitable potentials are applied, and that the resistance of the solution is kept relatively low. Beyond oxygen reduction, no other electrochemical processes (*e. g.* and H^+ ion discharge) are allowed to take place on the cathode. This can be controlled by plotting a current-voltage curve. Furthermore, one must avoid oxygen consumption occurring without current supply via local cell action (reaction between oxidizing-layer and ground metal). For the present, it appears possible with acidity values up to pH 4 in the electrolyte, to form cell combinations in which the intensity of the current varies

reliably and in proportion to the lowest values of the content of dissolved oxygen. The strength of the currents obtained is sufficient to be determined by usual amperemeters (small coil manometers such as the Gossen type, sensitivity 10^{-7} amperes, or a spot light galvanometer of the "Multiflex" type, sensitivity 8×10^{-9} amperes with an opening resistance of 300 Ohms). In resting solutions, with an O_2 content of 8 mg/1, 15 to 30 milliamperes per cm^2 electrode surface is obtained, and it is not difficult to measure 1/1000 of this value. In stirred or moved fluids, the current may increase up to 50 times this value. The independence of reducing substances is remarkable.

A current-voltage curve has been plotted by Tödt & Todt (1953) with different electrolytic oxygen contents 0.55 ml/1 and 7.8 mg/1, the electrolyte being composed of distilled water, with the addition of 300 mg Na_2SO_4/1 with a pH of 7, corresponding to the general biological values. The electrodes were of platinum and, to complete the cell, a calomel electrode with a potential against the normal hydrogen electrode of 0.285 volts (20° C) was used. It appeared that an oxygen diffusion current flows until — 950 millivolt. The area of the limit current is well pronounced, especially at low oxygen contents, the current being determined only by the oxygen arriving at the electrode. In other words, in this area the current is independent of the potential.

A perfectly constant anode-potential is most easily obtained by separating the anode from the electrolyte by means of a diaphragm, and by immersing it in a saturated KCl solution. To supply electrons for the reduction of the O_2 at the cathode, an unpolarisable anode of cast iron with a high carbon and copper content may be employed. Lead and zinc may also be used. The anode must be able to dissolve constantly, supplying as many electrons as the oxygen requires for its reduction. Both lead and zinc are especially suitable for resting biological fluids.

As measuring electrode, a polarisable cathode of platinum or an amalgamated gold electrode — which is more suitable for resting biological fluids — may be used. Evidently the formation of an oxygen adsorption layer on the amalgam electrode is better avoided due to the greater mobility of the

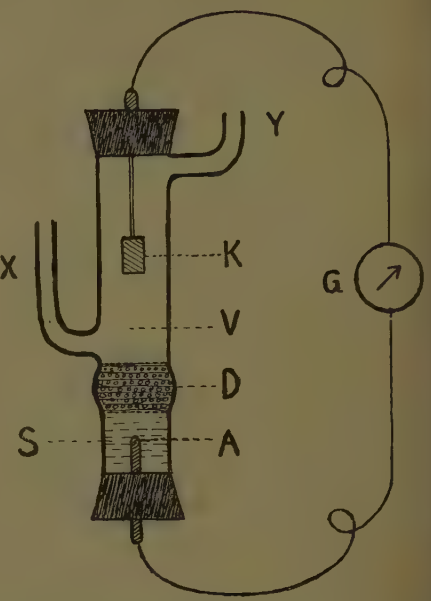
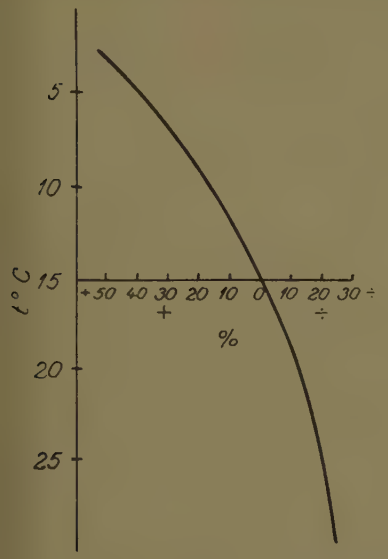


Fig. 1. The microapparatus for electroche
O_2 measurement.

mercury surface — a mobility which, of c
has to be understood in atomic dimensions, i
a constant change of the mercury atoms upo
surface.

The measurements are carried out wit
apparatus of the following construction.

The cathode (K) of 0.3 cm^2 amalgamated
gold is immersed in the liquid, the oxygen c
of which is to be measured. It forms the ga
cell with the lead (or zinc) anode (A) an
values are read on the galcanometer (G) in n
amperes. To avoid lead ions diffusing int
liquid, a diaphragm (D) glass-filter G3 o
separates the anode immersed in saturated
solution from the reaction medium. Further
2 per cent agar is added to the KCl solution,
coagulates and forms an agar-salt bridge.
the anode and cathode chambers have
completely filled with fluid. Fluid or gas
mitted through the tube X and drained throu
The measuring electrode must be freq
cleansed of grease and other impurities
trichlorethylene. The apparatus was made by
Jacobs Glasteknik, Copenhagen.



The graphic temperature correction curve (Ohle, 1953).

iediately after commencing measurements, rated values are found owing to the oxygen covering every metal (adsorption layer or d skin). These high values decrease very however. Constant values and complete f disturbance in the solution and deflection tained after about 5 minutes.

temperature will influence the results to iderable extent, all increase of temperature g the viscosity of the electrolyte, so that the d quantities of oxygen and the values of rent are increased. It is therefore important ie temperature be kept constant *e. g.* by the apparatus immersed in a water stat.

measurements give a temperature coefficient per cent per degree centigrade at room ature. By calculating the solubility of oxygen ying temperatures, the required correction e obtained by means of Ohle's graphic ature correction curve (1953).

n this curve one can calculate how many nt must be added or subtracted to the d in order to obtain the value corresponding calibration temperature (in this case 15° C). ological fluids, a correction of 2.5 per cent ree centigrade is to be preferred.

Calibration of the apparatus.

To control the resistance of the apparatus, and to establish that the measurements really take place in the oxygen diffusion area on the current-voltage curve, the two electrodes may be connected with a general voltmeter after the apparatus has been filled with water at 20° C. A value of —615 millivolt is then found. By directing pure oxygen through the water, the value increases to —590 millivolt. As is known, in polarographic measurements a fixed tension is applied upon the dropping electrode at —600 millivolt in order to arrive at the tension area where the diffusion current flows most pronouncedly during the oxygen determination. Using a voltage of —600 millivolt and thereabouts, the oxygen diffusion current may thus be rendered independent of the tension, and, as shown by Tödt, the current-voltage dependency only reoccurs at —950 millivolt, in consequence of hydrogen development being initiated. In other words, the cathode ought not to have a potential that there is no hydrogen current and attention be able to retain this potential constantly over a long period. Both of these conditions are fulfilled in the potential measurements reported here.

The calibration of the apparatus may be carried out in 3 different ways: 1) Chemical calibration by means of oxygen analysis, for instance by the the Winkler method with mangan-hydroxide. 2) Admission of gas mixtures with known oxygen content. 3) Calibration using an electrolyte solution of known oxygen content. The two latter methods are most simple to perform.

The first adjustment is made by reducing the electrolyte's oxygen content to as little as possible by admitting purified nitrogen from a tank. It is not absolutely necessary to begin the calibration with nil oxygen content, because completely pure nitrogen is difficult to obtain. One may therefore begin with some higher oxygen content — provided that there is no hydrogen current — and attention is paid to this fact, a parallel displacement through zero is made. In most cases, the value 0.7 per cent O_2 is used as the lowest obtainable oxygen content.

Thereafter, the current value at air saturation (21 per cent O_2) is determined, and finally — if necessary — the value on saturation with a random mixture, *e. g.* 12.5 per cent O_2. As the oxygen

content is directly proportional to the strength of the current, on may interpolate to obtain a rectilinear curve. Such a calibration curve for dissolved oxygen in a phosphate buffer at 20° C is shown in figure 3. This curve practically corresponds to the values of a physiological saline solution and of pure water. Investigations as to the influence of the conductivity of the electrolyte upon the oxygen diffusion current — in other words the influence of the dissociated salts present — have shown that it is solely dependent upon the size of the cathode (Tödt & Todt, 1953). A cathode diminished 100 times will decrease the current about 100 fold, but the resistance between the electrodes is only 10 times higher at the same time. This means that, if a 0.3 cm^2 gold amalgam electrode (or still smaller) is chosen, independence of salt content, and consequently of changing resistance, may be obtained.

If we assume ordinary barometric pressure, and an oxygen tension of 159 mm Hg, we may also calculated the calibration values in volume per cent dissolved oxygen, referring to the absorption coefficient in water. (By saturation with air we obtain for instance $2.4.\frac{159}{760} = 0.51$ volume per cent (38° C).

That the curve stops at 0.7 microampere may be attributed to the fact that the nitrogen cannot be completely cleared of oxygen. This factor

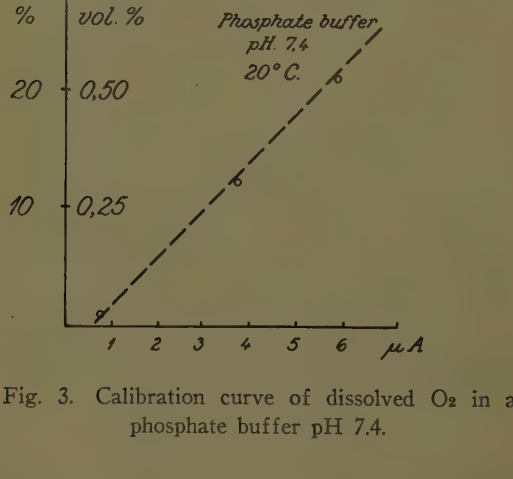


Fig. 3. Calibration curve of dissolved O_2 in a phosphate buffer pH 7.4.

seems to be so constant, however, that we accept the curve as sufficiently accurate practical purposes.

The above calibration curve is based up series of 25 measurements made partly wi simple coil galvanometer (Gossen type 83 sensibility 0—10 microamperes), and partly a spot- light galvanometer ("Multiflex" sensibility 8×10^{-9} ampere. Resistance 300 O

Some minutes are required before the comes to rest and finds a constant level. Sti or movement of the electrolyte will increase values, but if the apparatus is kept steady thermostat, constant values will be obt within 10 minutes (even within 5 minutes in fluids, *e. g.* urine). All the reported results c reproduced with a satisfactorily small erro 3—4 per cent. For comparison, reference ma made to the Roughton-Scholander volum method, whose experimental error is at 10—15 per cent.

The above series of measurements obtained partly with a lead anode, and p with a zinc anode. The most constant va seem to be given by the zinc anode.

DETERMINATION OF DISSOLVED OXYGEN IN SOME BIOLOGICAL FLUIDS

Saliva

Mixed human saliva may be colle either under stimulation by chewing para wax, or unstimulated at the earliest hours after the last meal. Saliva obtain greater quantities than 3 ml during minutes is described as stimulated.

For 25 saliva samples at 22° C, the va ascertained lay between 1.8 and 2.6 with a mean value of 2.1 μA. This responding to 0.13—0.24 volume per dissolved oxygen, or a mean value of volume per cent at 22° C and a genera pressure of 159 mm Hg. For unstimulated saliva, the values generally 15—20 per cent lower.

After admission of air (21 per cent O_2)

0 min.	15.0 μA	0 min.	14.0 μA	0 min.	15 μA	0 min.	12.5 μA
1 »	9.1 »	1 »	*8.7* »	1 »	8.9 »	1 »	7.8 »
2 »	8.0 »	2 »	7.9 »	5 »	6.1 »	3 »	6.5 »
3 »	7.6 »	5 »	6.5 »	10 »	*5.8* »	11 »	*5.6* »
4 »	7.2 »	8 »	6.1 »	15 »	5.7 »	15 »	5.8 »
5 »	6.6 »	12 »	*6.1* »				
0 »	6.1 »	14 »	6.1 »				
2 »	*5.4* »						
5 »	5.3 »						

Mean 5.7 μA ± 0.03 (25 experiments).

After admission of 0.7 per cent O_2 (nitrogen saturated)

0 min.	2.0 μA	0 min.	2.0 μA	0 min.	2.0 μA
1 »	1.5 »	1 »	1.32 »	1 »	1.13 »
2 »	1.4 »	3 »	1.05 »	3 »	1.05 »
6 »	1.14 »	10 »	0.85 »	6 »	0.95 »
0 »	*0.9* »	12 »	*0.93* »	10 »	*0.93* »

Mean 0.9 μA ± 0.04 (20 experiments).

After admission of a mixture of 12.5 per cent O_2.

0 min.	4.0 μA	0 min.	4.9 μA	0 min.	4.5 μA	0 min	5.0 μA
2 »	*3.3* »	2 »	3.7 »	2 »	3.9 »	2 »	4.0 »
5 »	2.7 »	5 »	*3.5* »	5 »	3.7 »	5 »	3.6 »
0 »	2.4 »	10 »	3.5 »	10 »	*3.6* »	10 »	*3.5* »

Mean 3.5 μA ± 0.03 (20 experiments).

he solubility coefficient of oxygen in at different temperatures is to be ired (with the apparatus in a thermo- we have to take due regard to above- oned Ohle correction diagram, and to ulate the values, subtracting 2.5 per per degree centigrade over room rature. As the measurements are d out in an air saturation of about m Hg the values correspond to this n tension.

ppears from the curve the solubility of n in saliva under general partial pres- s only one-third of that in water.

order to avoid the saliva foaming, drops of octyl-alcohol were added. appeared to have had no influence the readings. In control experiments other methods, *e. g.* Winkler's titrimetric method, salivary values varying between 0.10 and 0.35 volume per cent were found. With the Roughton-Scholander microgazanalyzer, the values varied between 0.10 and 0.45 volume per cent (Eggers Lura 1955).

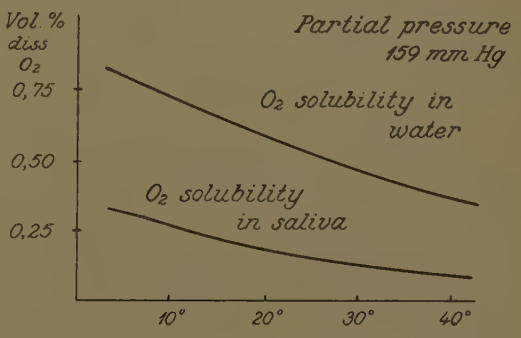


Fig. 4. O_2 solubility curves of water and saliva at different temperatures.

Given high bacterial content in the saliva, *i. e.* especially in the morning and fasting saliva, the values of dissolved oxygen are generally somewhat lower than later in the day, when the saliva has been stimulated and aired.

Milk

Warm milk transferred directly from the cow to the apparatus was used. Cooling and storage at room temperature leads to the oxygen content of the milk increasing rather quickly, and gradually it reaches values similar to those of water at room temperature, *i. e.* 0.40—0.50 volume per cent.

Fresh milk, however, contains only 0.22—0.28 volume per cent O_2 at 25° C. From measurements of the corresponding carbon acid content with the Scholander method, it appears that the carbonic acid content of milk is rather high just after milking, but decreases with cooling and storage the oxygen content increasing.

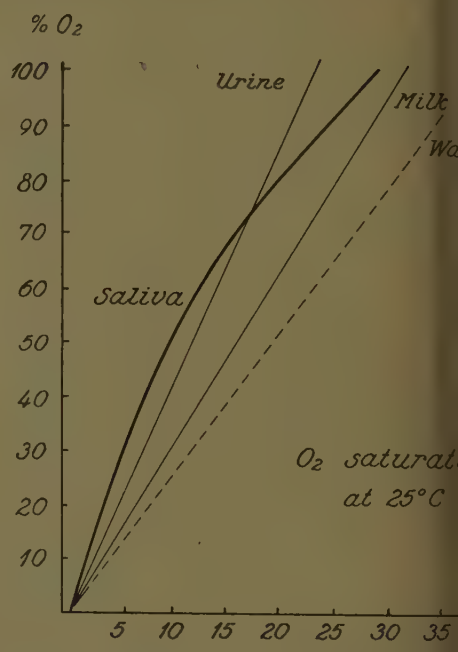


Fig. 5. Solubility curves of some fluids different oxygen mixtures.

It appears that the salivary curve dev somewhat from those of urine and mil

Urine

Most values for the oxygen content are somewhat below those of water. At 25° C, values of from 0.33 to 0.45 volume per cent were found. Generally the values of the urine are adjusted very quickly owing to the low viscosity of this fluid.

Solubility curves for the above-mentioned fluids with different oxygen mixtures, *e. g.* pure oxygen (100 per cent), air (21 per cent) and purified nitrogen (0.7 per cent), were determined. The gas mixtures were admitted to the liquids for 10 minutes at a constant temperature of 25° C.

Blood

Some measurements of the diss oxygen content of venous blood, withdrawn from the elbow vein, were made. Oxalate was added to the b which was transferred directly to apparatus at a temperature of 25° C. V corresponding to a dissolved oxygen c of about 0.25 volume per cent were p against the calibration curve for Even if the lower room temperature comparison with the body temperatur considered, the value found is too compared with those previously publish other authors for venous blood,

ally amount to 0.10 volume per cent
ved O_2.
haps the content of blood catalase
bs the electrochemical measurements
lood catalase content is 25—30 times
than that of saliva), and this fact
ly has to be considered as sufficient
ation for correction for, or inactivation
catalase. A vibrator for stirring the
during the measurements appeared to
nore stable values than measurements
ting fluid, and a practical solution of
roblem is now being examined. An
gement for the direct anaerobic trans-
blood to the apparatus is also under
igation.

SUMMARY

A mircoapparatus for the electrochemical measurement of dissolved oxygen in resting biological fluids in quantities of 3—4 ml is described. The values correspond to those found with other metrods, *e. g.* the volumetric and the chemical methods, and the results may be reproduced with an experimental error of about 3—4 per cent. Some examples of measurements of the oxygen content of saliva, milk and urine are given.

Supplementary experiments with venous blood have demonstrated that the measurements in resting fluid are inconstant and the method is not indicated in this case.

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ON THE DISAPPEARANCE OF ERYTHROCYTES

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(Received for publication December 28, 1954)

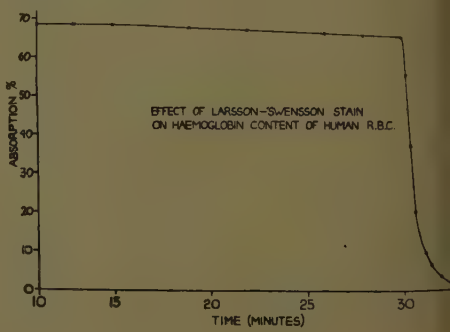
Larsson & Svensson (1952) have described a simple effective method of counting eosinophil granulocytes directly without recourse to stained films. Their method involves diluting the blood in the white blood cell pipette with a fluid of the following composition: —

Giemsa	5 ml
0.1 % aqueous eosin	25 ml
Propylene glycol	20 ml
Destilled water	50 ml

The propylene glycol causes a disappearance of the red cells from the suspension quite suddenly, 15—20 minutes after dilution of the blood. This greatly facilitates the enumeration and differentiation of the stained white blood cells in the counting chamber. It is the mechanism of this disappearance in which we became interested.

Larsson & Svensson state that no hemolysis takes place, and that the disappearance is due to the refractive index of red cells becoming the same as that of the suspending fluid. For the purpose of quantitative microspectrophotometry, we were attempting to match the refractive indices of mounting media to that of biological specimens with particular reference to erythrocytes.

Oxyhemoglobin has three absorption bands in the visible spectrum, the α at 575OA., the β at 542OA. and the γ or Soret band at 415OA. The absorption of the Soret band is ten times that of the other two. Erythrocytes viewed down a microscope in monochromatic light of 41 look virtually black. Such visual obse tion provides substantial proof that h lysis does occur when red cells are suspe in Larsson—Svensson fluid. The h absorbing erythrocytes suddenly be paler and disappear as hemoglobin is from the cells. We have measured hemoglobin content of an individual red as a function of time after suspe (See Fig.). As can be seen, hemolysis place extremely rapidly commencing about thirty minutes, and proceede completion until there was no diffe between the hemoglobin content of Ghost and that of the surrounding fluid, 'cell' was then invisible. The time before hemolysis occurred varied cons ably from cell to cell but, once started, al proceeded rapidly to completion. The phenomenon occurred when the Giemsa eosin were omitted from the fluid but be prevented by the addition of 0.15 sodium chloride.



explanation is that the red cell is permeable to propylene glycol which 15—20 minutes to diffuse into the d produce sufficient osmotic pressure ence to cause hemolysis.

SUMMARY

disappearance of red cells in the on—Swensson technique of eosinophil ng is due to hemolysis.

ACKNOWLEDGEMENTS

Our thanks are due to Professor J. M. Yoffey in whose laboratories this work was conducted, and for the grants obtained through him from the Rockefeller Foundation and the Medical Research Council.

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A SIMPLE METABOLISM CAGE FOR RATS

By R. A. SPIRES and A. B. MORRISON

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(Received for publication Dec. 12, 1954)

From the studies on the water and electrolyte metabolism of rats it was necessary to make accurate collections of the daily urinary output of single animals. It was found that the conventional metabolism cages were so large and the funnel used to collect urine exposed so great an area, that errors due to evaporation became significant.

For this investigation special metabolism cages were designed with Perspex funnels to fit the cages.

The cages were relatively simple and easily constructed. They were required to be:

(a) small, for use with single rats.
(b) easily set up and stored.
(c) quickly and easily cleaned.
(d) so constructed that the food was kept away from the urine.

The first requirement was satisfied by using a standard steriliser basket constructed in galvanised iron wire: the dimensions of the basket 15 cm × 15 cm × 15 cm, the walls were of 1.5 cm diamond mesh and the bottom 6 mm square mesh. A second bottom section provided the lid.

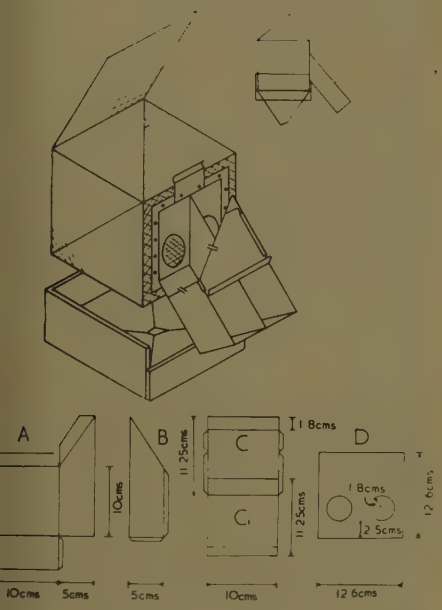
A holder for food and water was constructed from No. 22 gauge galvanised iron sheet. Sections of the sheet were cut in accordance with drawings A—D (Fig. 1). The main body (A) was formed with the securing flanges at the sides bent outwards, and the bottom one turned to form a hook on which the food holder pivoted during assembly. All joints except the actual fi of the holder to the cage were soldered

The cover for the food was made in sections: the slides for the sliding hatch formed by the lower half of the side fla the upper half securing the cover to the body.

Part of one wall of the cage was cu in the form of a 10 cm square, 2.5 cm each side. The lower flange on the body of the food holder was fitted ove lower section of the cage, and pivote this edge, until the upper flange on the came against the upper part of the wall. opening in the side of the cage was pletely closed by the food box, adjust of the securing flanges being necessa allow the holder to fit closely.

The inner plate (D) was placed insid cage, with the outlet holes to the food water holder at the lower level, and h through the mesh of the cage to the food Two of the bolts also held the fastene the lid of the cage.

The square funnel was made of Pe sheeting 1.5 mm thick with a suppo wall 4.5 mm thick. A sector of a cir 16.5 cm radius was cut which containe equilateral triangles lying edge to edg the centre a hole 2.5 cm was bored. hole prevented warping of the plate d bending and eventually formed the out the funnel.



t was applied along the "line" of each on of the triangles in turn and the x bent to form a square funnel with m sides. The joint was then sealed at which was preferable to using a compound, as it gave a more rounded , and one easier to clean.

wall section, 7.5 cm wide and 66 cm was cut and bent to give four sides, approximately 16.5 cm long. Here one seal only was required. A guide 2.5 cm high was marked all round ll.

en the joints had been completed the of the funnel section were ground lly and the corners rounded slightly he curved corners of the wall.

funnel was fitted into the wall section the guide line, leaving a wall of 5 cm into which the cage fitted. Sealing compound was poured into the groove and allowed to take up its position round the whole of the joint. A section of one wall 3.8 cm deep and 16.5 cm long was cut away, and formed a recess through which the food holder projected when the apparatus was set up. The funnel outlet was ground off to the requisite angle and size.

In this apparatus the area from which urine can evaporate is small and losses even

Table I. *Recoveries of water discharged into the funnel.*

Volume of water run in	Volume of water recovered
5.2 ml	5.0 ml
5.4 »	5.1 »
5.4 »	5.2 »
5.3 »	5.0 »

with small daily outputs were insignificant. The arrangements whereby the animal obtains food and water are such that the animal cannot urinate while searching for food, without the urine being discharged into the funnel.

The evaporation has been measured by setting up a burette containing water over the cage, so that water was delivered on to the funnel near its edge. The rate of emptying was adjusted so that the burette allowed about 5 ml of water to fall on to the funnel in 24 hours. The volume of water collected in 24 hours proved that there was an al quantitative recovery. The results of an experiment are set out in Table I. evident that losses of urine due to eva tion or occlusion on the surface of the fu are very small.

SUMMARY

The construction of a simple metab cage for the rat has been described. Rec of urine over 24 hours from one anim satisfactory.

PORPHYRIN CONCENTRATION FROM ULTRAVIOLET EXTINCTION

A NOTE ON THE CALCULATION

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(Received for publication Feb. 15, 1955)

orphyrins can be measured spectrometrically or fluorometrically. The est method of quantitative measurein a laboratory equipped with a modern electric spectrophotometer is spectrometric reading at the wavelength of oret band in acid solution as proposed einsson, Rimington & Barnes (1949) Rimington & Sveinsson (1950). There owever, at present some confusion concerning the calculation constants to be used.

The above-mentioned authors read the extinction of the porphyrins dissolved in dilute HCl at three wave-lengths, *i. e.*, the Soret maximum and further 380 and 430 mμ. From the readings obtained the corrected extinction is calculated by means of the following formulas:

(1) $d = 2\,e^{max} - (e^{430} + e^{380})$ (max = Soret maximum)

(2) $d^{corr} = d/k$ (k = correction divisor)

e correction divisors (k) given by gton & Sveinsson (1950) are 1.833 oproporphyrin, 1.844 for uroporphyrin .584 for protoporphyrin. In a recent let from Hilger & Watts, Ltd., Lonhese constants are, however, given as 1.835, 1.844, and 1.668 respectively, and in a personal communication Professor Rimington has confirmed that he now employs the latter values. The correct calculation constants are therefore:

(3) protoporphyrin k = 1.668 (in 5—25 per cent HCl)
coproporphyrin k = 1.835 (in 0.1—0.15 N HCl)
uroporphyrin k = 1.844 (in 0.5 N HCl)

extinctions are transformed to expresin μg per ml of solution in the cell by of the extinction constants for pure rations of porphyrins ($E^{1\,\%}_{1\,cm}$ = calextinction for a solution containing r 100 ml and read in a 1 cm layer). gton & Sveinsson (1950) employed llowing values for $E^{1\,\%}_{1\,cm}$: Coproporin 0.1—0.15 N HCl 8100 (from Jope & O'Brien, 1945); uroporphyrin I in 0.5 N HCl 6517 and the corresponding value for uroporphyrin III 6452 (their own measurements); protoporphyrin 4890 in 5—25 per cent HCl (from Grinstein & Winthrobe, 1948). But in the above-mentioned pamphlet from Hilger & Watts it is stated that the value for coproporphyrin is only 6667, as the 8100 value for Jope & O'Brien has not been confirmed, and this statement is confirmed by Rimington. The other constants are given as in Rimington &

pported by a grant from Statens almindelige absfond (Danish State Science Fund).

Sveinsson's paper. The values of $E_{1\,cm}^{1\,\%}$ (1 g per 100 ml) correspond to the following figures of $d_{1\,cm}^{corr}$ for solutions containing 1 μg per ml (= 10^{-4} g per 100 ml): Protoporphyrin $4890/10^4 = 0.489$; coproporphyrin $6667/10^4 = 0.6667$; uroporphyrin I $6517/10^4 = 0.6517$; uroporphyrin III 0.6452.

If we employ 0.6535 as an approximate value for uroporphyrin, these calculation constants may be expressed as follows:

Protoporphyrin:

$d_{1\,cm}^{corr} = 0.489$ corresponds to 1 μg per ml,

Coproporphyrin:

$d_{1\,cm}^{corr} = 0.667$ corresponds to 1 μg per ml,

Uroporphyrin:

$d_{1\,cm}^{corr} = 0.653$ corresponds to 1 μg per ml,

i. e., per ml of solution in the cell of spectrophotometer.

In the paper of Askevold (1951) the responding figures are, however, give 0.65 for coproporphyrin and 0.50 for porphyrin. The former value is close t correct one while the latter is too low consequently gives too high values for porphyrin *(i. e.,* 0.65/0.50 times too h This value of Askevold is presumably b on the provisional value of $E_{1\,cm}^{1\,\%}$ for porphyrin used by Sveinsson, Rimingt Barnes (1949) which was 5,000. Becaus method of Askevold is used fairly e sively in Scandinavia it is importan draw attention of the erroneous calcul constants given.

The constants are easier to emplo routine analysis when presented in the lowing way:

$$\text{Protoporphyrin } \mu\text{g per ml} = \frac{d_{1\,cm}}{0.489 \cdot 1.668} = 1.225 \cdot d_{1\,cm}$$

$$\text{(4)} \quad \text{Coproporphyrin } \mu\text{g per ml} = \frac{d_{1\,cm}}{0.667 \cdot 1.835} = 0.817 \cdot d_{1\,cm}$$

$$\text{Uroporphyrin } \mu\text{g per ml} = \frac{d_{1\,cm}}{0.653 \cdot 1.844} = 0{\cdot}831 \cdot d_{1\,cm}$$

SUMMARY

The porphyrin concentration is calculated from the spectrophotometric readings at the maximum in the near ultraviolet (Soret band), 380 and 430 mμ, by means of the equations (1) and (2). The readings are performed in 2—25 % HCl for protoporphyrin, 0.1—0.15 N HCl for coproporphyrin and 0.5 N HCl for uroporphyrin. The values for the calculation divisor k are given (3). From the corrected $d_{1\,cm}$ the porphyrin concentration of the solution in the cell is calculated by means of the equations (4).

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Letters to the Editors

ETERMINATIONS OF GLANDULAR MUCOPROTEIN IN THE GASTRIC JUICE OF PATIENTS WITH PERNICIOUS TAPEWORM ANAEMIA

rnberg (1936, 1941, 1947) has demon-
d the presence of intrinsic factor (IF)
e gastric juice (GJ) of patients with
cious tapeworm anaemia (PTA) by
istering their GJ together with extrinsic
to patients with cryptogenetic perni-
anaemia (PA). A low production of
PTA has been suggested, but no quan-
e determination of the IF secretion has
performed.

til recently the quantitative determina-
of IF has been almost impossible, the
criterion of IF being the response of a
patient. The response of PA patients,
ver, shows much individual variation.
losage is also intricate; excessive doses
ting the intestinal uptaking of vitamin
Glass, Boyd, Stephanson & Jones,
.

ss and coworkers have shown that one
onent of GJ, glandular mucoprotein
P), is associated with IF activity, and
he occurrence and secretory pattern of
wo in various diseases show paral-
. Thus, regardless of whether GMP
e IF or not, it seems possible to gather
nation on the IF secretion by deter-
g GMP.

order to evaluate the IF secretion in
GMP was determined according to the
d of Glass (1953) together with HCl in
sting GJ samples and the fractional
neal samples obtained at 15 min. inter-
after the intravenous injection of
6 units of insulin. The peak value for
was usually found in the fraction ob-
tained 45 min. after the insulin injection, the HCl peak coming later. The peak values were distributed as seen in the table. In three cases a fasting sample was obtained, but only one contained GMP (69 mg/100 ml).

The GMP secretion seems to exhibit parallelism with gastroscopic findings (Siurala, 1955) and the uropepsin excretion (Lumme, Mustakallio, Telkkä & Töttermann, 1954), and is consistent with the view of von Bonsdorff (1953) that a low production of IF may contribute to, but is not obligatory for, the development of PTA.

Further work is in progress to study the possible inhibitory effect of the worm on the secretion of GMP by also determining GMP :1. after the anaemic state is relieved by parenteral B_{12} administration and 2. after the expulsion of the worm. No indication of an inhibitory effect has been found in the few cases studied.

T a b l e 1. *Peak values obtained after insulin stimulation.*

Case No.	Glandular mucoprotein mg/100 ml	HCl mEq per liter
1	292	160
2	260	154
3	126	88
4	122	55
5	90	14
6	86	0
7	72	0
8	64	100
9	28	0
10	0	0
11	0	0

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Address:
IV Medical Clinic
Maria Hospital,
Helsingfors, Finland.

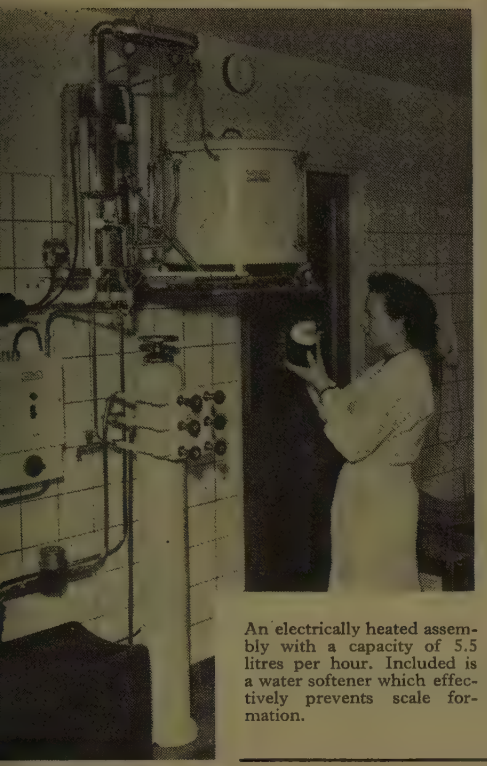
Ralph Gräsbeck

Erratum.

Platelet accelerator: Identical to proaccelerin and adsorbed from plasma?

A serious misprint in the letter to the Editor (Vol. 7, No. 1, p. 9) under the above title should be corrected. Line 13 from above, first column, should read:

. . . . platelet activity accounts for about *six* per cent . . . .



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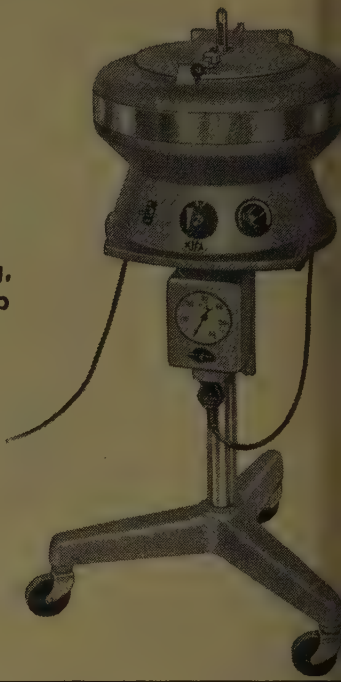

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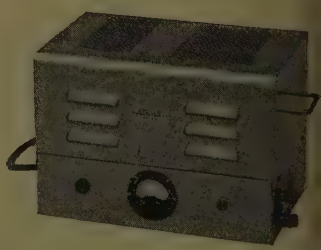

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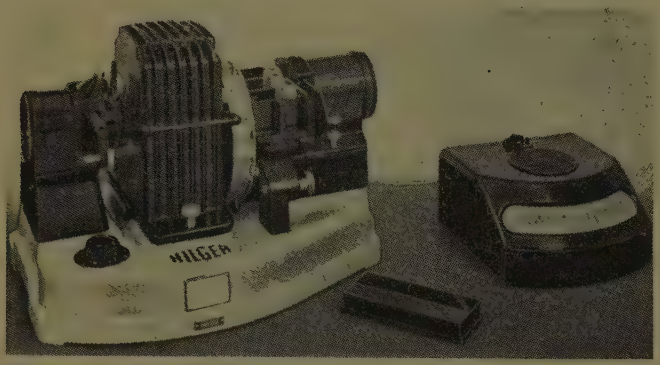

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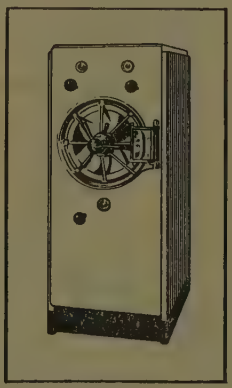

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